Use of Substituted 4-Biarylbutyric and 5-Biarylpentanoic Acid Derivatives for the Treatment of Multiple Sclerosis

Field

This invention relates to the use of enzyme inhibitors, and more particularly, to new and known matrix metalloprotease-inhibiting 4-Biarylbutyric Acids and 5-Biaryl-penta-noic Acids and derivatives thereof, for the prevention and treatment of Multiple Sclerosis.

Background

Multiple Sclerosis (MS) is a severely disabling disease which is characterized by focal inflammatory brain lesions. The major site of immunological attack is the myelin sheath formed by oligodendrocytes. As a consequence myelin, which is essential for fast conduction of nerve cell signals, is destroyed and oligodendrocytes eventually die.

Current therapies (βIFNs, Copaxone) and those currently in clinical development focus on modulation or suppression of the inflammatory processes involved. However, there is still a high medical need for more convenient and, in particular, more efficacious treatments.

Matrix metalloproteinases (MMPs) are a large family of Zn^{2+} endopeptidases that include 72 kDa (MMP-2) and 92 kDa gelatinase (MMP-9), collagenases (MMP-1, MMP-8, MMP-13), stromelysins 1-3 (MMP-3, MMP-10, MMP-11), matrilysin (MMP-7), macrophage metalloelastase (MMP-12) and membrane-bound MMPs 1-4. They are expressed in inflammatory conditions and are collectively capable of degrading most connective tissue macromolecules.

Substituted 4-Biarylbutyric and 5-Biarylpentanoic Acid Derivatives as Matrix Metalloprotease Inhibitors are described in WO 96/15096, WO 97/43237, WO 97/43238, WO 97/43239, WO 97/43240, WO 97/43245, WO97/43247 and WO 98/22436.

Hydroxamic acid derivatives with non-selective MMP-inhibitory activities were shown to be therapeutically efficacous in different animal models of Multiple Sclerosis (*J. Neuroimmunol.* 1997, *74*, 85-94; *Ann. Neurol.* 1998, *44*, 35-46).

Summary

This invention relates to the use for the prevention and treatment of Multiple Sclerosis of compounds having matrix metallprotease inhibitory activity of the generalized formula (I) :

$$(T)_XA\text{-}B\text{-}D\text{-}E\text{-}CO_2H\ .\qquad (I)$$

In the above generalized formula (I), $(T)_XA$ represents a substituted or unsubstituted aromatic 6-membered ring or heteroaromatic 5 - 6 membered ring containing 1 - 2 atoms of N, O, or S. T represents one or more substituent groups, the subscript x represents the number of such substituent groups, and A represents the aromatic or heteroaromatic ring, designated as the A ring or A unit. When N is employed in conjunction with either S or O in the A ring, these heteroatoms are separated by at least one carbon atom.

The substituent group(s) T are independently selected from the group consisting of halogen; alkyl; haloalkyl; haloalkoxy; alkenyl; alkynyl; $-(CH_2)_pQ$ in which p is 0 or an integer of 1 - 4; -alkenyl-Q in which the alkenyl moiety comprises 2 - 4 carbons; and alkynyl-Q in which the alkynyl moiety comprises 2 - 7 carbons. Q in the latter three groups is selected from the group consisting of aryl, heteroaryl, -CN, -CHO,

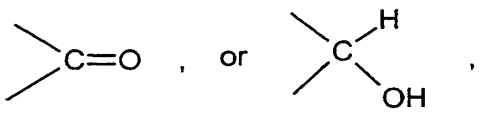
$-NO_2$, $-CO_2R^2$, $-OCOR^2$, $-SOR^3$, $-SO_2R^3$, $-CON(R^4)_2$, $-SO_2N(R^4)_2$, $-COR^2$, $-N(R^4)_2$, $-N(R^2)COR^2$, $-N(R^2)CO_2R^3$, $-N(R^2)CON(R^4)_2$, $-CHN_4$, $-OR^4$, and $-SR^4$.

In these formulae R^2 represents H, alkyl, aryl, heteroaryl, arylalkyl, or heteroaryl-alkyl. R^3 represents alkyl, aryl, heteroaryl, arylalkyl, or heteroaryl-alkyl. R^4 represents H; alkyl; aryl; heteroaryl; arylalkyl; heteroaryl-alkyl; alkenyl; alkynyl; alkyleneoxy, polyalkyleneoxy, alkylenethio or alkyleneamino terminated with H, alkyl, or phenyl; haloalkyl; lower alkoxycarbonyl; or acyl. When two R^4 groups are situated on a nitrogen, they may be joined by a bond to form a heterocycle, such as, for example, a morpholine, thiomorpholine, pyrrolidine, or piperidine ring.

Unsaturation in a moiety which is attached to Q or which is part of Q is separated from any N, O, or S of Q by at least one carbon atom. The A ring may be unsubstituted or may carry up to 2 substituents T. Accordingly, the subscript x is 0, 1, or 2.

In the generalized formula (I), B represents a bond or an optionally substituted aromatic 6-membered ring or a heteroaromatic 5 - 6 membered ring containing 1 - 2 atoms of N, O, or S. When B is a ring, it is referred to as the B ring or B unit. When N is employed in conjunction with either S or O in the B ring, these heteroatoms are separated by at least one carbon atom. There may be 0 -2 substituents T on ring B.

In the generalized formula (I), D represents



in which R^2 is defined as above and each R^2 may be the same or different.

In the generalized formula (I), E represents a chain of n carbon atoms bearing m substituents R^6, in which the R^6 groups are independent substituents, or constitute

spiro or nonspiro rings. Rings may be formed in two ways: a) two groups R^6 are joined, and taken together with the chain atom(s) to which the two R6 group(s) are attached, and any intervening chain atoms, constitute a 3 - 7 membered ring, or b) one group R^6 is joined to the chain on which this one group R^6 resides, and taken together with the chain atom(s) to which the R^6 group is attached, and any intervening chain atoms, constitutes a 3 - 7 membered ring. The number n of carbon atoms in the chain is 2 to 4, and the number m of R^6 substituents is an integer of 1 - 3.

Each group R^6 is independently selected from the group consisting of:

*fluorine;

*hydroxyl, with the proviso that a single carbon atom may bear no more than one hydroxyl group;

*alkyl;

*aryl;

*heteroaryl;

*arylalkyl;

*heteroaryl-alkyl;

*alkenyl;

*aryl-substituted alkenyl;

*heteraryl-substituted alkenyl;

*alkynyl;

*aryl-substituted alkynyl;

*heteroaryl-substituted alkynyl;

*-$(CH_2)_tR^7$, wherein t is 0 or an integer of 1 - 5 and

R^7 is selected from the group consisting of:

*N-phthalimidoyl;

*N-(1,2-naphthalenedicarboximidoyl);

*N-(2,3-naphthalenedicarboximidoyl);

*N-(1,8-naphthalenedicarboximidoyl);

*N-indoloyl;

*N-(2-pyrrolodinonyl);

*N-succinimidoyl;

*N-maleimidoyl;

*3-hydantoinyl;

*1,2,4-urazolyl;

*amido;

*urethane;

*urea;

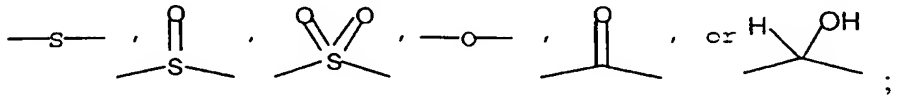
*nonaromatic substituted or unsubstituted heterocycles containing and connected through a N atom, and comprising one or two additional N, O, S, SO, or SO_2, and containing zero, one or two carbonyls, and optionally bearing a fused benzene or pyridine ring;

*amino;

*corresponding heteroaryl moieties in which the aryl portion of an aryl-containing R^7 group comprises 4 - 9 carbons and at least one N, O, or S heteroatom;

*-$(CH_2)_vZR^8$ in which v is 0 or an integer of 1 - 4, wherein

Z represents



;

and

R^8 is selected from the group consisting of:

*alkyl;

*aryl;

*heteroaryl;

*arylalkyl;

*heteroaryl-alkyl; and

*-C(O)R^9 in which R^9 represents alkyl of at least two carbons, aryl, heteroaryl, arylalkyl, or heteroaryl-alkyl;

and with the further provisos that

- when R^8 is -C(O)R^9 , Z is S or O;
- when Z is O, R^8 may also be alkyleneoxy or polyalkyleneoxy terminated with H, alkyl, or phenyl; and

*trialkylsilyl-substituted alkyl.

Furthermore, aryl or heteroaryl portions of any of the T or R^6 groups optionally may bear up to two substituents selected from the group consisting of -$(CH_2)_yC(R^4)(R^3)OH$, -$(CH_2)_yOR^4$, -$(CH_2)_ySR^4$, -$(CH_2)_yS(O)R^4$, -$(CH_2)_yS(O)_2R^4$, -$(CH_2)_ySO_2N(R^4)_2$, -$(CH_2)_yN(R^4)_2$, -$(CH_2)_yN(R^4)COR^{12}$, -$OC(R^4)_2O$- in which both oxygen atoms are connected to the aryl ring, $(CH_2)_yCOR^4$, -$(CH_2)_yCON(R^4)_2$, -$(CH_2)_yCO_2R^4$, -$(CH_2)_yOCOR^4$, -halogen, -CHO, -CF_3, -NO_2, -CN, and -R^3, in which y is 0 - 4. R^3 and R^4 are defined as above; in addition, any two R^4 which are attached to one nitrogen may be joined to form a heterocycle such as morpholine, thiomorpholine, pyrrolidine, or a piperidine ring.

Pharmaceutically acceptable salts of these compounds as well as commonly used prodrugs of these compounds such as O-acyl derivatives of invention compounds which contain hydroxy groups are also within the scope of the invention.

In addition to the above-described compounds, the invention also relates to pharmaceutical compositions having matrix metalloprotease inhibitory activity, which compositions comprise a compound of the invention as described above and in more detail in the detailed description below, and a pharmaceutically acceptable carrier.

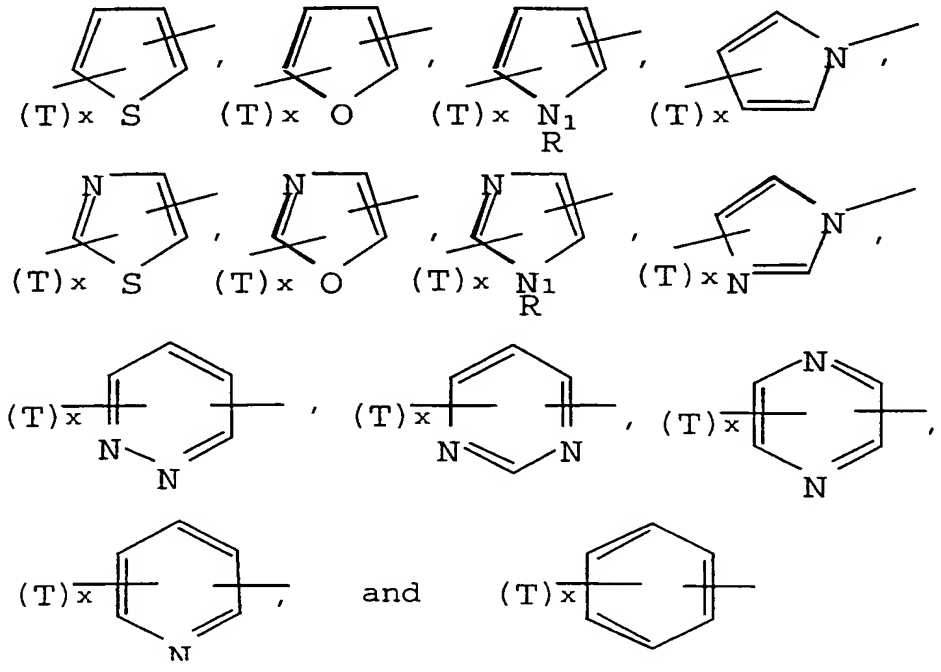
The invention also relates to a method of treating a mammal such as a human, a farm animal, or a domestic pet, to achieve an effect, in which the effect is treatment and prevention of Multiple Sclerosis.

Detailed Description

Surprisingly it has been found that particularly useful for the use for the prevention and treatment of Multiple Sclerosis are compounds having matrix metalloprotease inhibitory activity of the generalized formula:

$$(T)_XA\text{-}B\text{-}D\text{-}E\text{-}CO_2H \qquad (I)$$

in which $(T)_XA$ represents a substituted or unsubstituted aromatic or heteroaromatic moiety selected from the group consisting of:



in which R^1 represents H or alkyl of 1 - 3 carbons.

In these structures, the aromatic ring is referred to as the A ring or A unit, and each T represents a substituent group, referred to as a T group or T unit. Substituent groups T are independently selected from the group consisting of: the halogens -F, -Cl, -Br, and -I; alkyl of 1 - 10 carbons; haloalkyl of 1 - 10 carbons; haloalkoxy of 1 - 10 carbons; alkenyl of 2 - 10 carbons; alkynyl of 2 - 10 carbons; $-(CH_2)_pQ$ in which p is 0 or an integer 1 - 4; -alkenyl-Q in which the alkenyl moiety comprises 2 - 4 carbons; and -alkynyl-Q in which the alkenyl moiety comprises 2 - 7 carbons. Q in each of the latter three groups is selected from the group consisting of aryl of 6 - 10 carbons; heteroaryl comprising 4 - 9 carbons and at least one N, O, or S heteroatom; -CN; -CHO; $-NO_2$; $-CO_2R^2$; $-OCOR^2$; $-SOR^3$; $-SO_2R^3$; $-CON(R^4)_2$; $-SO_2N(R^4)_2$; $-C(O)R^2$; $-N(R^4)_2$; $-N(R^2)COR^2$; $-N(R^2)CO_2R^3$; $-N(R^2)CON(R^4)_2$; $-CHN_4$; $-OR^4$; and $-SR^4$. The groups R^2, R^3, and R^4 are defined as follows.

R^2 represents H; alkyl of 1 - 6 carbons; aryl of 6 - 10 carbons; heteroaryl comprising 4 - 9 carbons and at least one N, O, or S heteroatom; arylalkyl in which the aryl portion contains 6 - 10 carbons and the alkyl portion contains 1 - 4 carbons; or heteroaryl-alkyl in which the heteroaryl portion comprises 4 - 9 carbons and at least one N, O, or S heteroatom and the alkyl portion contains 1 - 4 carbons.

R^3 represents alkyl of 1 - 4 carbons; aryl of 6 - 10 carbons; heteroaryl comprising 4 - 9 carbons and at least one N, O, or S heteroatom; arylalkyl in which the aryl portion contains 6 - 10 carbons and the alkyl portion contains 1 - 4 carbons; or heteroaryl-alkyl in which the heteroaryl portion comprises 4 - 9 carbons and at least one N, O, or S heteroatom and the alkyl portion contains 1 - 4 carbons.

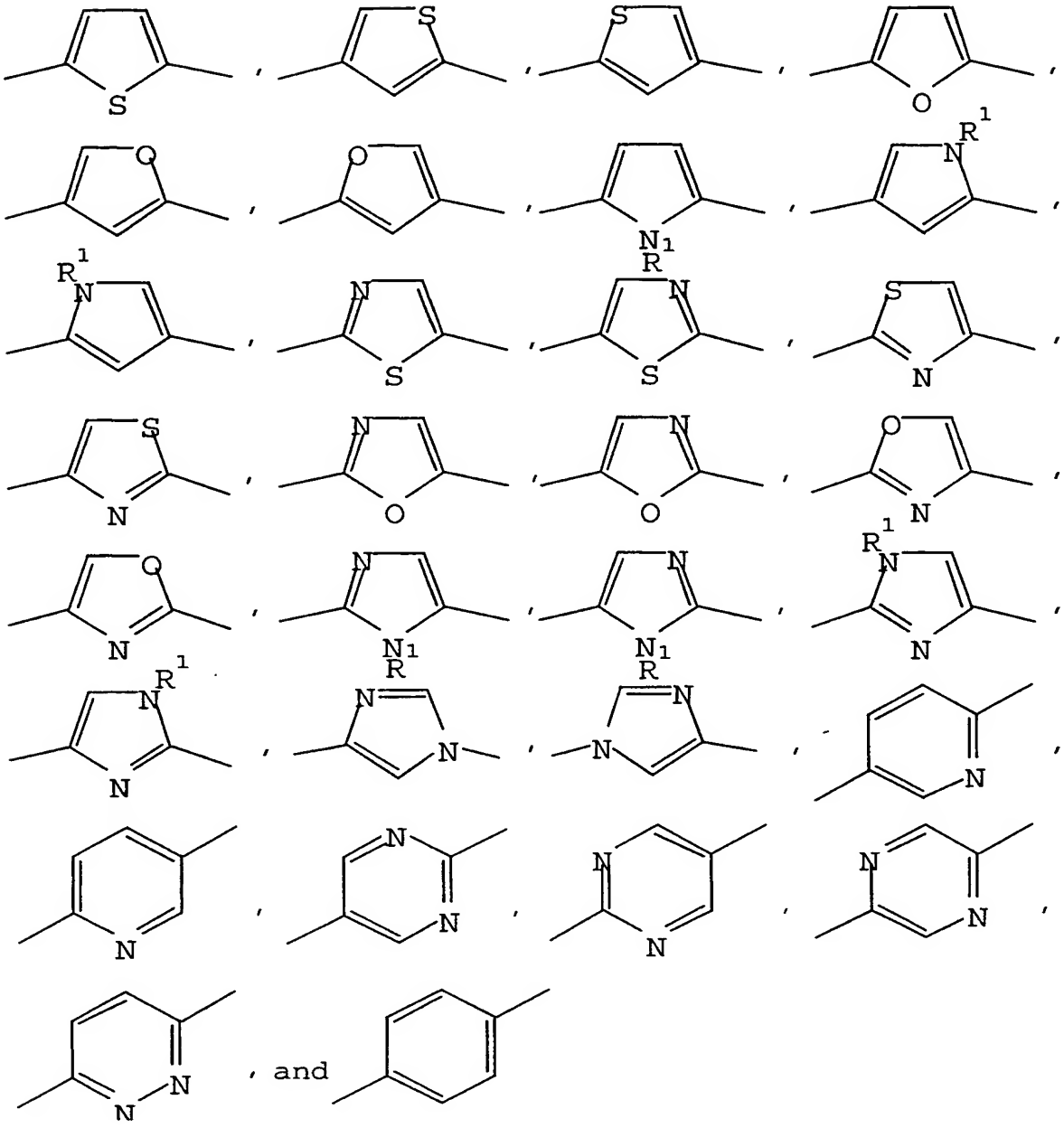
R^4 represents H; alkyl of 1 - 12 carbons; aryl of 6 - 10 carbons; heteroaryl comprising 4 - 9 carbons and at least one N, O, or S heteroatom; arylalkyl in which the aryl portion contains 6 - 10 carbons and the alkyl portion contains 1 - 4 carbons; heteroaryl-alkyl in which the heteroaryl portion comprises 4 - 9

carbons and at least one N, O, or S heteroatom and the alkyl portion contains 1 - 4 carbons; alkenyl of 2 - 12 carbons; alkynyl of 2 - 12 carbons; $-(C_qH_{2q}O)_rR^5$ in which q is 1-3, r is 1 - 3, and R^5 is H provided q is greater than 1, or R^5 is alkyl of 1 - 4 carbons, or phenyl; alkylenethio terminated with H, alkyl of 1-4 carbons, or phenyl; alkyleneamino terminated with H, alkyl of 1-4 carbons, or phenyl; $-(CH_2)_sX$ in which s is 1-3 and X is halogen; $-C(O)OR^2$; or $-C(O)R^2$.

When two R^4 groups are situated on a nitrogen, they may be joined by a bond to form a heterocycle, such as, for example, a morpholine, thiomorpholine, pyrrolidine, or piperidine ring.

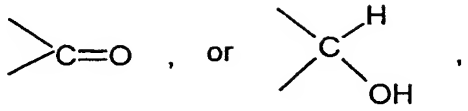
Any unsaturation in a moiety which is attached to Q or which is part of Q is separated from any N, O, or S of Q by at least one carbon atom, and the number of substituents, designated x, is 0, 1, or 2.

In the generalized formula (I), B represents a bond or an optionally substituted aromatic or heteroaromatic ring selected from the group consisting of:



in which R^1 is defined as above and each R^1 may be the same or different. These rings are referred to as the B ring or B unit. There may be 0-2 substituents T on the B ring, T being defined as above.

In the generalized formula (I), D represents the moieties



In the generalized formula (I), E represents a chain of n carbon atoms bearing m substituents R^6, referred to as R^6 groups or R^6 units. The R^6 groups are independent substituents, or constitute spiro or nonspiro rings. Rings may be formed in two ways: a) two groups R^6 are joined, and taken together with the chain atom(s) to which the two R6 group(s) are attached, and any intervening chain atoms, constitute a 3 - 7 membered ring, or b) one group R^6 is joined to the chain on which this one group R^6 resides, and taken together with the chain atom(s) to which the R^6 group is attached, and any intervening chain atoms, constitutes a 3 - 7 membered ring. The number n of carbon atoms in the chain is 2 or 3, and the number m of R^6 substituents is an integer of 1 - 3.

Each group R^6 is independently selected from the group consisting of the substituents listed below as items 1) - 16).

1) fluorine;

2) hydroxyl, with the proviso that a single carbon atom may bear no more than one hydroxyl group;

3) alkyl of 1 - 10 carbons;

4) aryl of 6 - 10 carbons;

5) heteroaryl comprising 4 - 9 carbons and at least one N, O, or S heteroatom;

6) arylalkyl in which the aryl portion contains 6 - 10 carbons and the alkyl portion contains 1 - 8 carbons;

7) heteroaryl-alkyl in which the heteroaryl portion comprises 4 - 9 carbons and at least one N, O, or S heteroatom, and the alkyl portion contains 1 - 8 carbons;

8) alkenyl of 2 - 10 carbons;

9) aryl-alkenyl in which the aryl portion contains 6 - 10 carbons and the alkenyl portion contains 2 - 5 carbons;

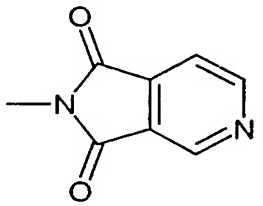
10) heteroaryl-alkenyl in which the heteroaryl portion comprises 4 - 9 carbons and at least one N, O, or S heteroatom and the alkenyl portion contains 2 -5 carbons;

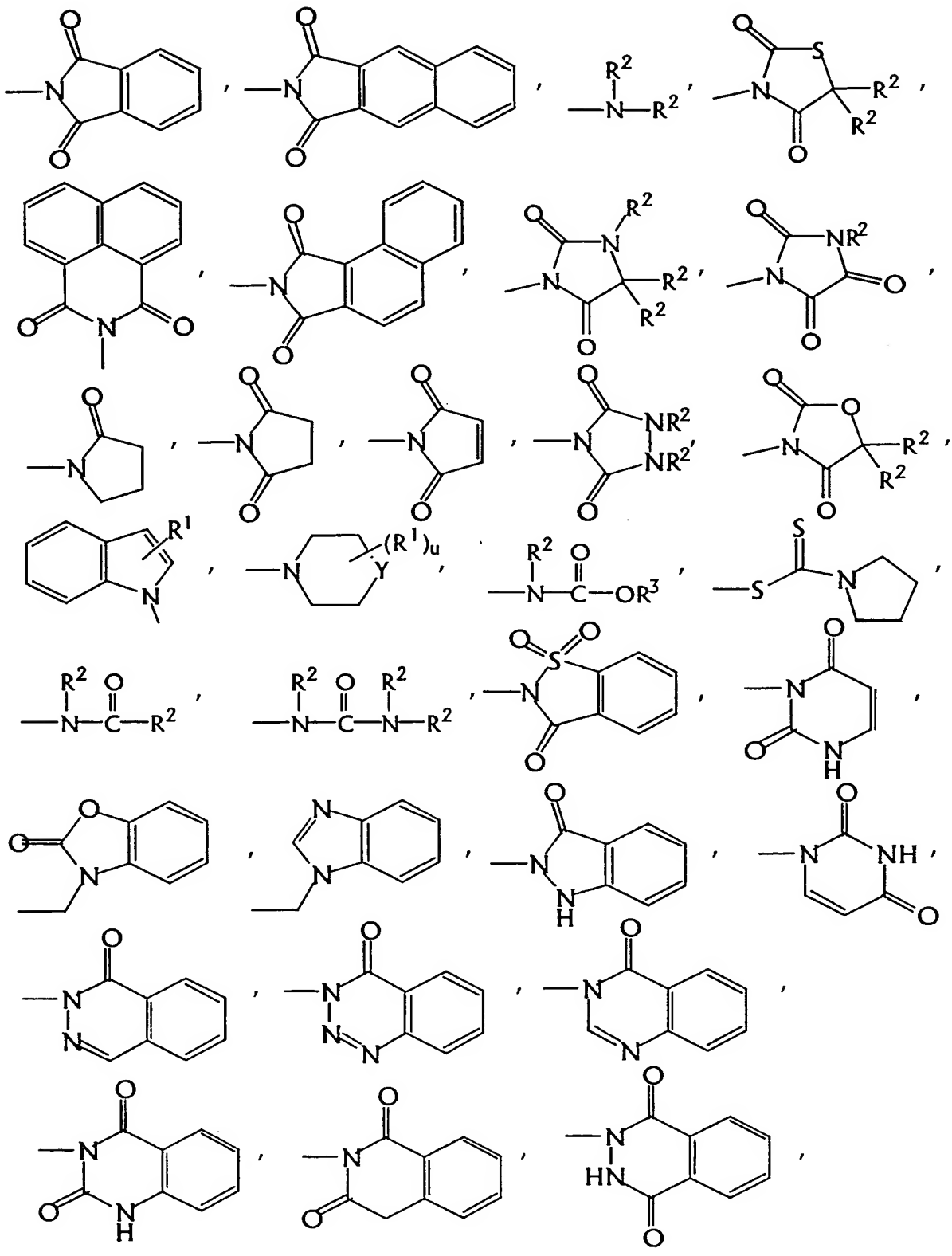
11) alkynyl of 2 - 10 carbons;

12) aryl-alkynyl in which the aryl portion contains 6 - 10 carbons and the alkynyl portion contains 2 - 5 carbons;

13) heteroaryl-alkynyl in which the heteroaryl portion comprises 4 - 9 carbons and at least one N, O, or S heteroatom and the alkynyl portion contains 2 - 5 carbons;

14) $-(CH_2)_tR^7$ in which t is 0 or an integer of 1 - 5 and R^7 is selected from the group consisting of





as well as corresponding heteroaryl moieties in which the aryl portion of an aryl-containing R^7 group comprises 4 - 9 carbons and at least one N, O, or S heteroatom. In such R^7 groups, Y represents O or S; R^1, R^2, and R^3 are as defined above, and each R^1, R^2 andR^3 may be the same or different; and u is 0, 1, or 2;

15) $-(CH_2)_vZR^8$ in which v is 0 or an integer of 1 to 4; Z represents -S-, -S(O)-, $-SO_2$-, -O-, carbonyl, or -CH(OH)-; and R^8 is selected from the group consisting of: alkyl of 1 to 12 carbons; aryl of 6 to 10 carbons; heteroaryl comprising 4 - 9 carbons and at least one N, O, or S heteroatom; arylalkyl in which the aryl portion contains 6 to 10 carbons and the alkyl portion contains 1 to 4 carbons; heteroaryl-alkyl in which the aryl portion comprises 4 - 9 carbons and at least one N, O, or S heteroatom and the alkyl portion contains 1 - 4 carbons; $-C(O)R^9$ in which R^9 represents alkyl of 2 - 6 carbons, aryl of 6 - 10 carbons, heteroaryl comprising 4 - 9 carbons and at least one N, O, or S heteroatom, or arylalkyl in which the aryl portion contains 6 - 10 carbons or is heteroaryl comprising 4 - 9 carbons and at least one N, O, or S heteroatom, and the alkyl portion contains 1 - 4 carbons, with the provisos that
- when R^8 is $-C(O)R^9$, Z is -S- or -O-;
- when Z is -O-, R^8 may also be $-(C_qH_{2q}O)_rR^5$ in which q, r, and R^5 are as defined above;

16) $-(CH_2)_wSi(R^{10})_3$ in which w is an integer of 1 to 3, and R^{10} represents alkyl of 1 to 4 carbons.

In addition, aryl or heteroaryl portions of any of the T or R^6 groups optionally may bear up to two substituents selected from the group consisting of $-(CH_2)_yC(R^4)(R^3)OH$, $-(CH_2)_yOR^4)$, $-(CH_2)_ySR^4)$, $-(CH_2)_yS(O)R^4)$, $-(CH_2)_yS(O)_2R^4)$, $-(CH_2)_ySO_2N(R^4))_2$, $-(CH_2)_yN(R^4))_2$, $-(CH_2)_yN(R^4))COR^3$, $-OC(R^4))_2O$- in which both oxygen atoms are connected to the aryl ring, $-(CH_2)_yCOR^4$, $-(CH_2)_yCON(R^4))_2$, $-(CH_2)_yCO_2R^4)$, $-(CH_2)_yOCOR^4)$, -halogen,

-CHO, -CF_3, -NO_2, -CN, and -R^3, in which y is 0 - 4; R^3 is defined as above; R^4 is defined as above, and each R^3 or R^4 may be the same or different and in addition, any two R^4 which are attached to one nitrogen may be joined to form a heterocycle, such as a morpholine, thiomorpholine, pyrrolidine, or piperidine ring.

Pharmaceutically acceptable salts of these compounds as well as commonly used prodrugs of these compounds such as O-acyl derivatives of these compounds are also within the scope of the invention.

In the compounds of the invention, the following are preferred.

The substituent group T, when it is on the ring A, is preferably halogen, 1-alkynyl-Q, or an ether OR^4 wherein R^4 is preferably alkyl of 1 - 12 carbons or arylalkyl in which the aryl portion is 6 - 10 carbons and the alkyl portion contains 1 - 4 carbons. Most preferably, T is halogen, or $-C\equiv C-(CH_2)_tOH$ in which t is an integer of 1 - 5, and when T is OR^4, R^4 is alkyl of 1 - 6 carbons, or benzyl.

The subscript x, which defines the number of T substituents, is preferably 1 or 2, most preferably 1, and this substituent T is preferably on the 4- position of ring A.

The A ring is preferably a phenyl or thiophene ring, most preferably phenyl. The A ring preferably bears at least one substituent group T, preferably located on the position furthest from the position of the A ring which is connected to the B ring.

The B moiety of generalized formula (I) is a bond or a substituted or unsubstituted aromatic or heteroaromatic ring, in which any substituents are groups which do not cause the molecule to fail to fit the active site of the target enzyme, or disrupt the relative conformations of the A and B rings, such that they would be detrimental. Such groups may be, but are not limited to, moieties such as lower alkyl, lower alkoxy, CN, NO_2, halogen, etc. The B moiety is preferably a 1,4-phenylene or 2,5-thiophene ring, most preferably 1,4-phenylene.

The compounds according to the invention can exist in stereoisomeric forms which either behave as image and mirror image (enantiomers), or which do not behave as image and mirror image (diastereomers). The invention relates both to the enantiomers or diastereomers and their respective mixtures. These mixtures of the enantiomers and diastereomers can be separated into the stereoisomerically uniform constituents in a known manner.

The D unit is most preferably a carbonyl or a -CHOH- group.

The group R^6 is preferably:

1) arylalkyl wherein the aryl portion contains 6 - 10 carbons and the alkyl portion contains 1 - 8 carbons;

2) $-(CH_2)_tR^7$ wherein t is 0 or an integer of 1 - 5 and R^7 is an imidoyl group fused to an aromatic residue, or the 1,2,3-benzotriazin-4(3H)-one-3-yl group; or

3) $-(CH_2)_vZR^8$ wherein v is 0 or an integer of 1 - 4 , Z is S or O, and R^8 is aryl of 6 - 10 carbons or arylalkyl wherein the aryl portion contains 6 to 12 carbons and the alkyl portion contains 1 to 4 carbons.

The group R^6 is most preferably one of the following, and in these, any aromatic moiety is preferably substituted:

1) arylalkyl wherein the aryl portion is phenyl and the alkyl portion contains 1 - 4 carbons;

2) $-(CH_2)_tR^7$ wherein t is an integer of 1 - 3, and R^7 is N-phthalimidoyl, 1,2,3-benzotriazin-4(3*H*)-one-3-yl, N-(1,2-naphthalenedicarboximidoyl), N-(2,3-naphthalenedicarboximidoyl), or N-(1,8-naphthalenedicarboximidoyl); or

3) $-(CH_2)_vZR^8$ wherein v is an integer of 1 - 3, Z is S, and R^8 is phenyl.

It is to be understood that as used herein, the term "alkyl" means straight, branched, cyclic, and polycyclic materials. The term "haloalkyl" means partially or fully halogenated alkyl groups such as $-(CH_2)_2Cl$, $-CF_3$ and $-C_6F_{13}$, for example.

In one of its embodiments, the invention relates to compounds of generalized formula (I) in which at least one of the units A, B, T, and R^6 comprises a heteroaromatic ring. Preferred heteroaromatic ring-containing compounds are those in which the heteroaryl groups are heteroaryl of 4 - 9 carbons comprising a 5 - 6 membered heteroaromatic ring containing O, S, or NR^1 when the ring is 5-membered, and N when said ring is 6-membered. Particularly preferred heteroaromatic ring-containing compounds are those in which at least one of the A and B units comprises a thiophene ring. When A unit is thiophene, it is preferably connected to B unit at position 2 and carries one substituent group T on position 5. When B Unit is thiophene, it is preferably connected through positions 2 and 5 to D and A units respectively.

In another embodiment, the invention relates to compounds of generalized formula (I), in the E unit of which n is 2 and m is 1. These compounds thus possess two carbon atoms between the D unit and carboxyl group, and carry one substituent on this two-carbon chain.

In another of its embodiments, the invention relates to compounds of generalized formula (I) in which the A ring is a substituted or unsubstituted phenyl group, the B ring is p-phenylene, and aryl portions of any aryl-containing T and R^6 moieties

contain only carbon in the rings. These compounds thus contain no heteroaromatic rings.

In another of its embodiments, the invention relates to compounds of generalized formula (I) in which m is 1 and R^6 is an independent substituent. These compounds are materials which contain only a single substituent R^6 on the E unit, and this substituent in not involved in a ring.

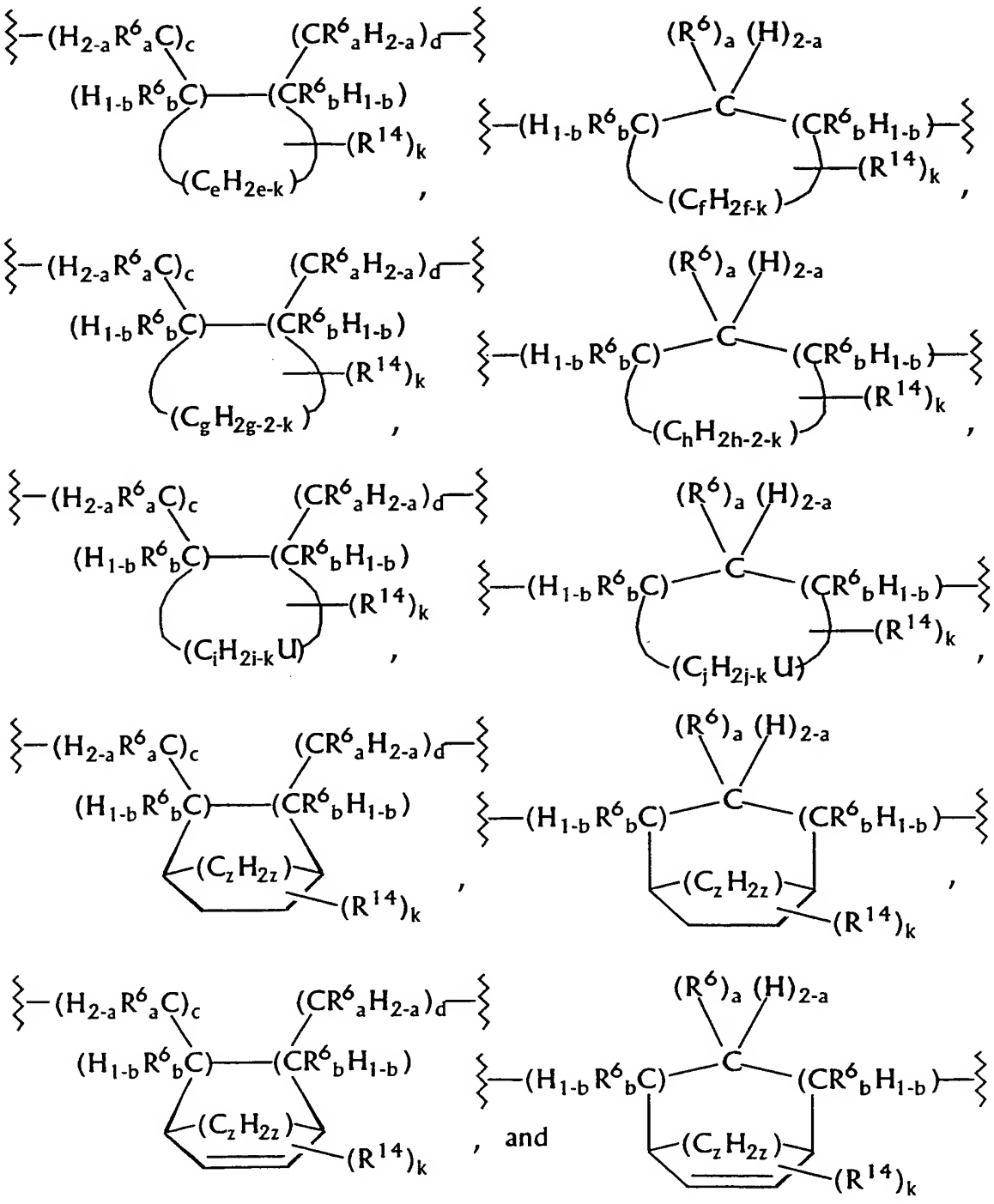
Preferred compounds of general formula (I) in which R^6 is $-(CH_2)_tR^7$ have t as an integer of 1-5. Preferred compounds of general formula (I) in which R^6 is $-(CH_2)_vZR^8$ have v as an integer of 1-4 and Z as -S- or -O-. Preferred compounds of general formula (I) in which R^6 is alkyl contain 4 or more carbons in said alkyl and those in which R^6 is arylalkyl contain 2-3 carbons in the alkyl portion of said aryl-alkyl.

In another of its embodiments, the invention relates to compounds of generalized formula (I) in which the number of substituents m on the E unit is 2 or 3; and when m is 2, both groups R^6 are independent substituents, or together constitute a spiro ring, or one group R^6 is an independent substituent and the other constitutes a spiro ring; and when m is 3, two groups R^6 are independent substituents and one group R^6 constitutes a ring, or two groups R^6 constitute a ring and one group R^6 is an independent substituent, or three groups R6 are independent substituents. This subset therefore contains compounds in which the E unit is di- or trisubstituted, and in the disubstituted case any rings formed by one or both R^6 groups are spiro rings, and in the trisubstituted case, the R^6 groups may form either spiro or nonspiro rings.

In another of its embodiments, the invention relates to compounds of generalized formula (I) in which the number of substituents m on the E unit is 1 or 2; and when m is 1, the group R^6 constitutes a nonspiro ring; and when m is 2, both groups R^6 together constitute a nonspiro ring or one group R^6 is an independent substituent and the other constitutes a nonspiro ring. This subset therefore contains compounds in

which the E unit carries one or two substituents R^6, and at least one of these substituents is involved in a nonspiro ring.

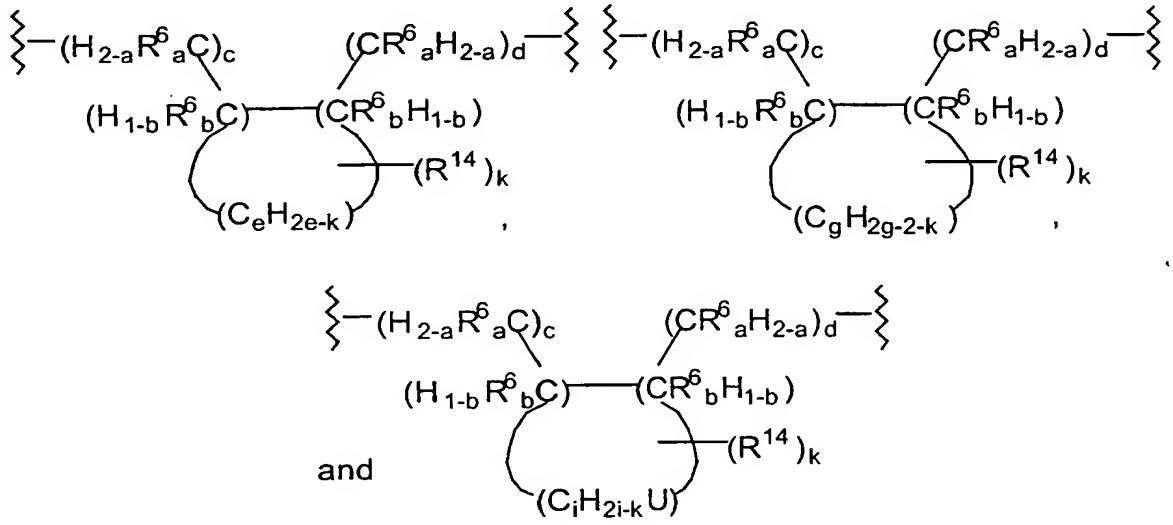
More particularly, representative compounds of generalized formula (I) in which one or more of the substituent groups R^6 are involved in formation of nonspiro rings have E units of the following structures:



in which a is 0, 1, or 2; b is 0 or 1; c is 0 or 1; d is 0 or 1; c + d is 0 or 1; e is 1 - 5; f is 1 - 4; g is 3 - 5; h is 2 - 4; i is 0 - 4; j is 0 - 3; k is 0 - 2; the total number of groups R^6 is 0, 1, or 2; U represents O, S, or NR^1; and z is 1 or 2; Each group R^{14} is independently selected from the group consisting of: alkyl of 1 - 9 carbons; arylalkyl

in which the alkyl portion contains 1 - 7 carbons and the aryl portion contains 6 - 10 carbons; alkenyl of 2 - 9 carbons; aryl-substituted alkenyl in which the alkenyl portion contains 2 - 4 carbons and the aryl portion contains 6 - 10 carbons; alkynyl of 2 - 9 carbons; aryl-substituted alkynyl in which the alkynyl portion contains 2 - 4 carbons and the aryl portion contains 6 - 10 carbons; aryl of 6 - 10 carbons; $-COR^2$; $-CH(OH)R^2$; $-CO_2R^3$; $-CON(R^2)_2$; $-(CH_2)_tR^7$ in which t is 0 or an integer of 1 - 4; and $-(CH_2)_vZR^8$ in which v is 0 or an integer of 1 to 3, and Z represents -S-, S(O), SO_2 , or -O-. R^1, R^7, and R^8 have been defined above.

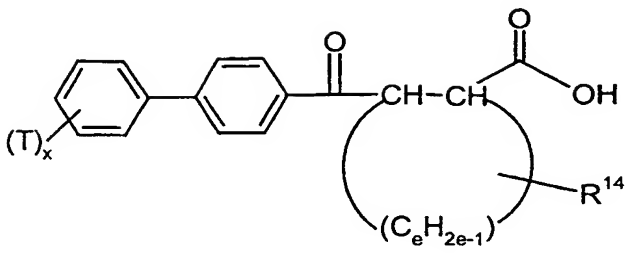
Preferred compounds of generalized formula (I) in which one or more of the substituent groups R^6 are involved in formation of nonspiro rings have E units of the following structures:



and

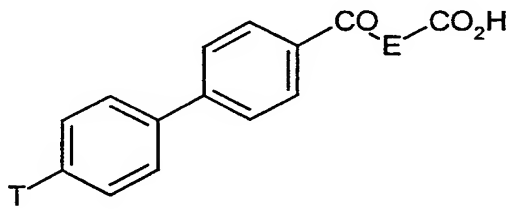
in which a, b, c, d, (c + d), e, g, i, k, the total number of groups R^6, U, and R^{14} are as defined above.

The more preferred compounds for the use for the prevention and treatment of Multiple Sclerosis of generalized formula (I), in which one or more of the substituent groups R^6 are involved in formation of nonspiro rings have the formula



in which the subscript x is 1 or 2; one substituent T is located on the 4-position of the A ring, relative to the point of attachment between the A and B rings; e is 2 or 3; and R^{14} is as defined above.

Very particularly preferred is the use of the compounds of the general formula (I')



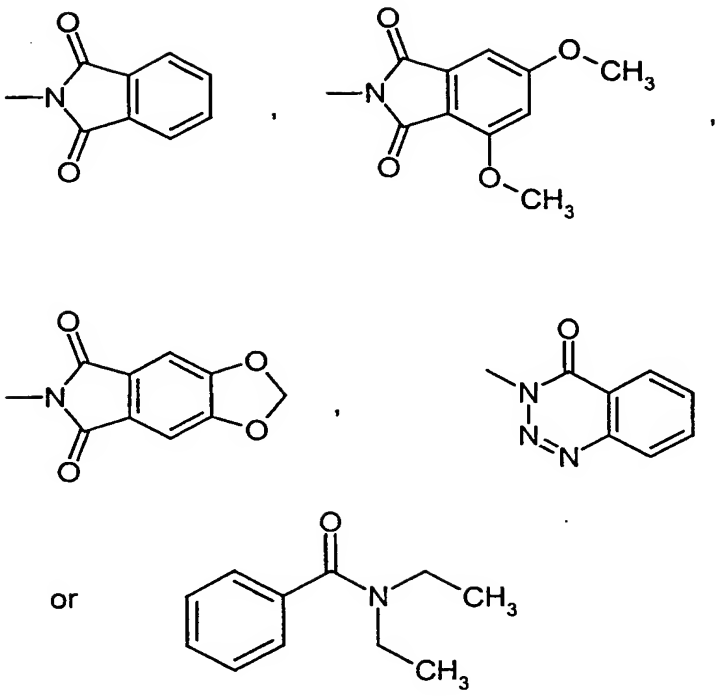
(I')

wherein

T is (C_1-C_4)-alkyl, (C_1-C_4)-alkoxy, chloride, bromide, fluoride, acetoxy, hydroxy, cyano, trifluoromethyl or trifluoromethoxy,

CO-E-CO_2H represents a 3-carboxyl-5-R^7-pentan-1-on-1-yl- or a 2-carboxyl-3-(R^7-methyl)-cyclopentan-1-yl)carbonyl-residue, wherein

R^7 represents a group of the formula

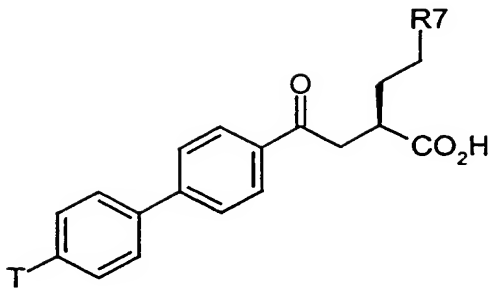


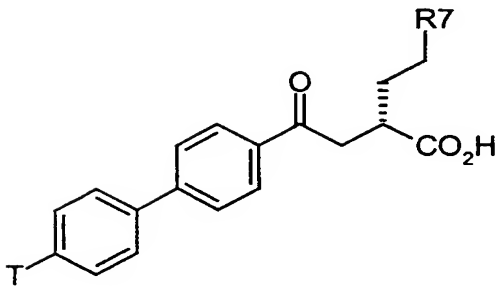
and their salts.

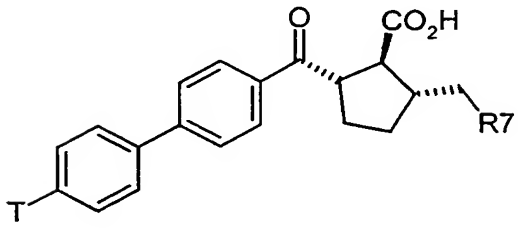
In case of the cyclopentane derivatives the trans, trans-diastereomers are preferred.

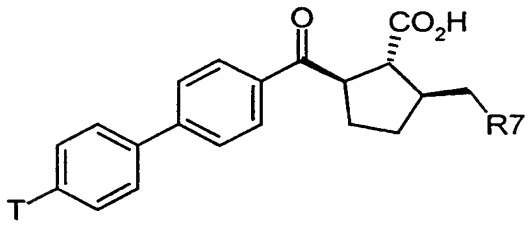
Furthermore preferred is the use of such enantiomers of pairs of enantiomers at a chiral center adjacent to the carboxylic acid moiety of the group of the formula CO-E-CO_2H in compounds of the general formula (I') that more potently inhibit MMP-2 and/or MMP-9.

The pairs of enantiomers can be exemplified by the following structures:



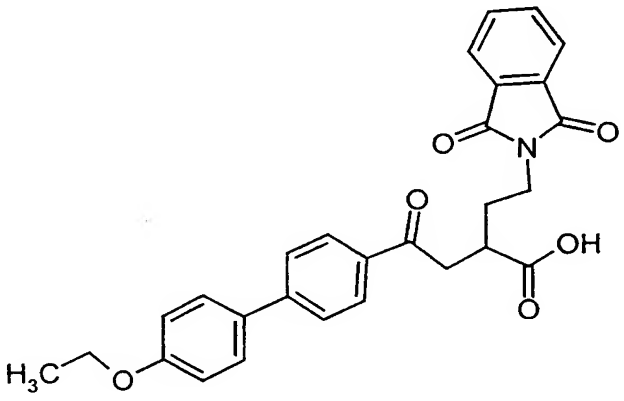




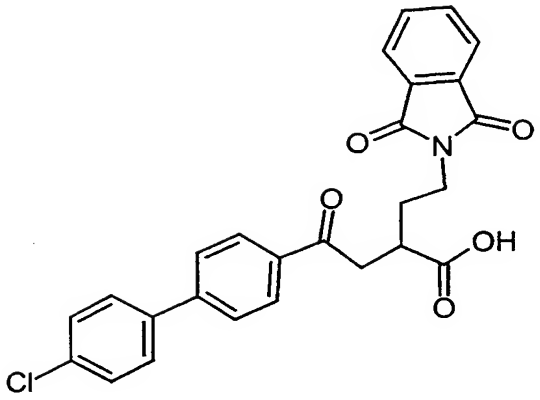


Especially preferred is the use of the following compounds:

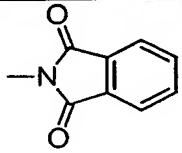
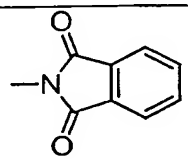
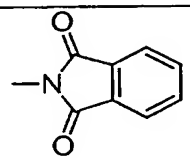
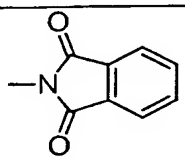
(+)-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(4'-ethoxy[1,1'-biphenyl]-4-yl)-4-oxobutanoic acid,

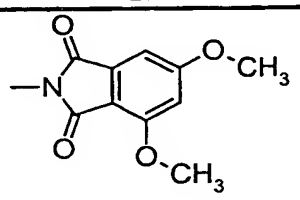
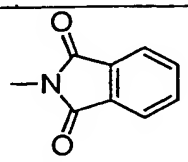
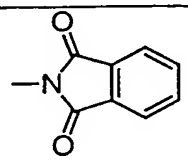
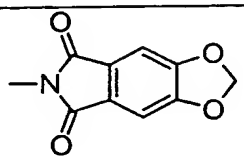
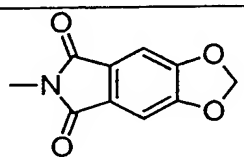
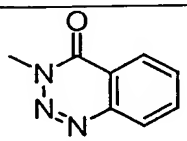
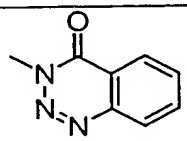


(+)-4-(4'-chloro[1,1'-biphenyl]-4-yl)-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-oxobutanoic acid



In another aspect of the invention the following new compounds of the general formula (I') are provided, wherein CO-E-CO_2H represents a 3-carboxyl-5-R^7-pentan-1-on-1-yl-residue, and wherein T and R^7 have the meaning indicated in the following table:

T	R^7	racemate, (+)- or (-)-enantiomer	
OEt		(+)	;
OEt		(-)	;
OAc		rac	;
OH		rac	;

T	R^7	racemate, (+)- or (-)-enantiomer	
Cl		rac	;
Br		(+)	;
Br		(-)	;
Cl		(+)	;
Cl		(-)	;
CN		rac	or
OCF_3		rac	.

General Preparative Methods:

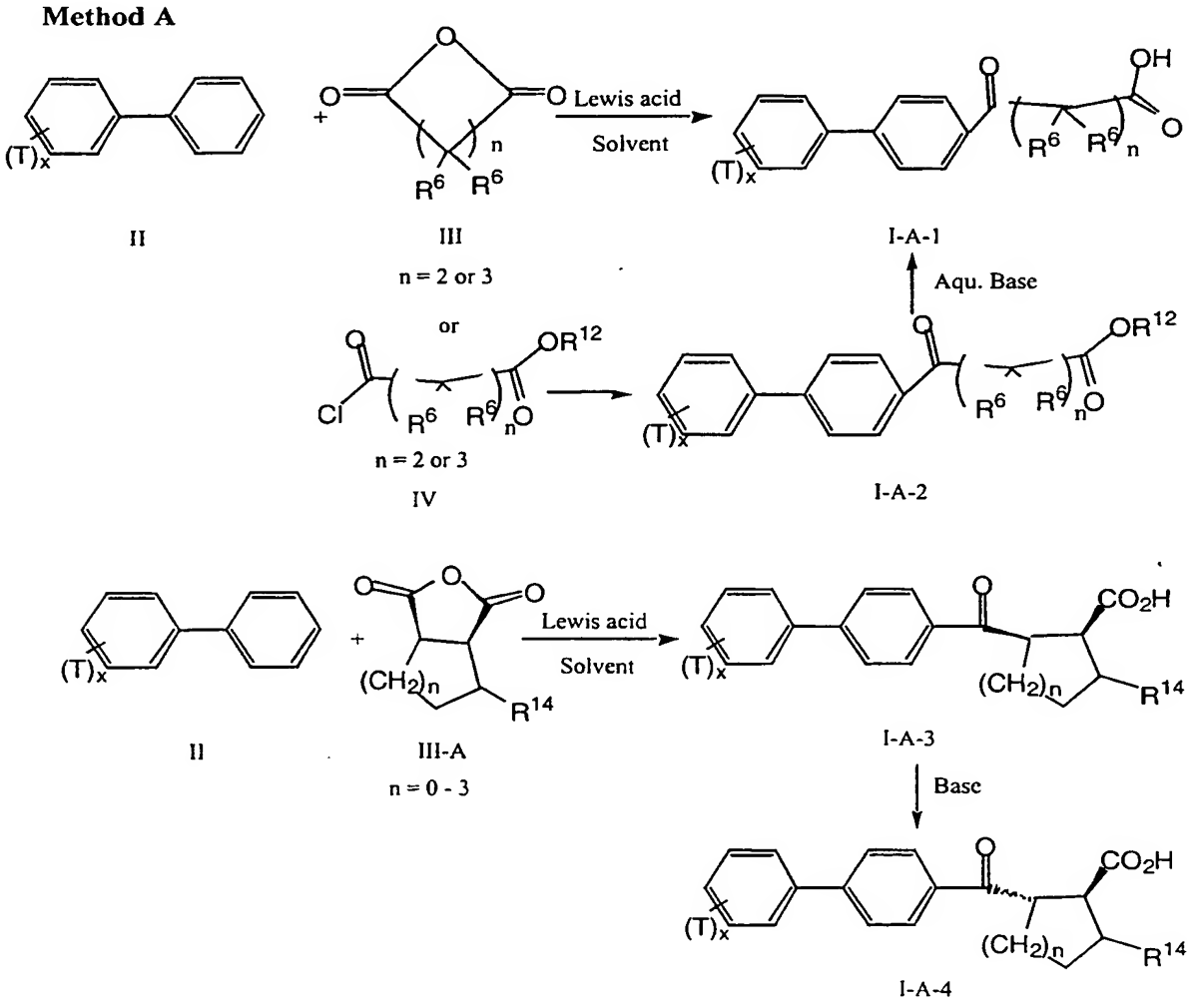
The compounds of the invention may be prepared by use of known chemical reactions and procedures as described in details in WO 96/15096, WO 97/43237, WO 97/43238, WO 97/43239, WO 97/43240, WO 97/43245, WO97/43247 and WO 98/22436. Nevertheless, the following general preparative methods are presented to aid the reader in synthesizing the inhibitors. General methods A through K may be used to prepare appropriately substituted 4-biaryl-4-oxobutanoic acids, 4-aryl-4-oxo-butanoic acids, 5-biaryl-5-oxopentanoic acids, or 5-aryl-5-oxopentanoic acids. These general methods are also found in WO 96/15096 along with exemplary preparations of the keto acids. The choice of a specific synthetic method is dictated by the proviso that the conditions used do not effect undesired changes in the T or R^6 moieties of the compounds prepared.

All variable groups of these methods are as described in the generic description if they are not specifically defined below. The variable subscript n is independently defined for each method. When a variable group with a given symbol (i.e. R^6 or T) is used more than once in a given structure, it is to be understood that each of these groups may be independently varied within the range of definitions for that symbol. As defined above, the compounds of the invention contain as the E unit a chain of 2 or 3 carbon atoms bearing 1 to 3 substituents R^6 which are not defined as H. By contrast, it is to be noted that in the general method schemes below, the R^6 groups are used as if their definition includes H, to show where such R^6 groups may exist in the structures, and for ease in drawing. No change in the definition of R^6 is intended by this non-standard usage, however. Thus, only for purposes of the general method schemes below, R^6 may be H in addition to the moieties set forth in the definition of R^6. The ultimate compounds contain 1 to 3 non-hydrogen groups R^6.

General Method A - The key intermediates in which the rings A and B are substituted phenyl and phenylene respectively are conveniently prepared by use of a Friedel-Crafts reaction of a substituted biphenyl II with an activated acyl- containing

intermediate such as the succinic or glutaric anhydride derivative III or acid chloride IV in the presence of a Lewis acid catalyst such as aluminum trichloride in an aprotic solvent such as 1,1,2,2-tetrachloroethane. The well known Friedel-Crafts reaction can be carried out with many alternative solvents and acid catalysts as described by E. Berliner, *Org. React.*, 5, 229 (1949) and H. Heaney, *Comp. Org. Synth.*, 2, 733 (1991).

Method A



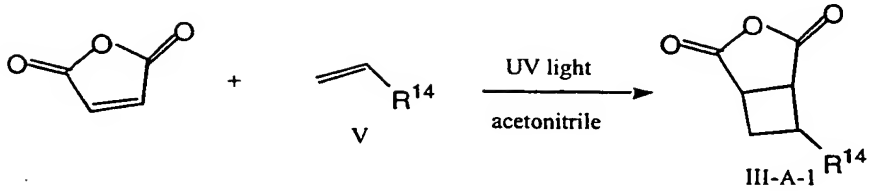
If the anhydride III is monosubstituted or multiplesubstituted in an unsymmetrical way, the raw product I-A often exists as a mixture of isomers via attack of the anhydride from either of the two carbonyls. The resultant isomers can be separated into

pure forms by crystallization or chromatography using standard methods known to those skilled in the art.

When they are not commercially available, the succinic anhydrides III can be prepared via a Stobbe Condensation of a dialkyl succinate with an aldehyde or ketone (resulting in side chain R^6), followed by catalytic hydrogenation, hydrolysis of a hemiester intermediate to a diacid and then conversion to the anhydride III by reaction with acetyl chloride or acetic anhydride. Alternatively, the hemiester intermediate is converted by treatment with thionyl chloride or oxalyl chloride to the acid chloride IV in which R^{12} is lower alkyl. For a review of the Stobbe condensation, including lists of suitable solvents and bases see W.S. Johnson and G.H. Daub, *Org. React.*, **6**, 1 (1951). This method, as applied to the preparation of III (R^6 = H, isobutyl and H, n-pentyl), has been described by D. Wolanin, et al., US Patent 4,771,038, Sep. 13, 1988.

Method A is especially useful for the preparation of cyclic key intermediates such as I-A-3 in which two R^6 groups are connected in a methylene chain to form a 4-7 membered ring. Small ring (3-5 member) anhydrides are readily available only as cis isomers which yield cis invention compounds I-A-3. The trans compounds I-A-4 are then prepared by treatment of I-A-3 with a base such as DBU in THF.

The substituted four member ring starting material anhydrides such as III-A-1 are formed in a photochemical 2+2 reaction as shown below. This method is especially useful for the preparation of compounds in which R^{14} is acetoxy or acetoxymethylene. After the subsequent Friedel-Crafts reaction the acetate can be removed by basic hydrolysis and the carboxyl protected by conversion to 2-(trimethylsilyl)ethyl ester. The resultant intermediate with R^{14} = CH_2OH can be converted to key intermediates with other R^{14} groups by using procedures described in General Method K.



The Friedel Crafts method is also useful when double bonds are found either between C-2 and C-3 of a succinoyl chain (from maleic anhydride or 1-cyclopentene-1,2-dicarboxylic anhydride, for example) or when a double bond is found in a side chain, such as in the use of itaconic anhydride as starting material to yield products in which two R^6 groups as found on one chain carbon together form an exo-methylene ($=CH_2$) group. Subsequent uses of these compounds are described in Methods D and E.

General Method B - Alternatively key intermediates can be prepared via a reaction sequence involving mono-alkylation of a dialkyl malonate VI with an alkyl halide to form intermediate VII, followed by alkylation with a halomethyl biphenyl ketone VIII to yield intermediate IX. Compounds of structure IX are then hydrolyzed with aqueous base and then heated to decarboxylate the malonic acid intermediate and yield I-B-2 (Method B-1). By using one equivalent of aqueous base the esters I-B-2 with R^{12} as alkyl are obtained, and using more than two equivalents of base the acid compounds (R^{12} = H) are obtained. Optionally, heat is not used and the diacid or acid-ester I-B-1 is obtained. Alternatively, the diester intermediate IX can be heated with a strong acid such as concentrated hydrochloric acid in acetic acid in a sealed tube at about 110 °C for about 24 hr to yield I-B-2 (R^{12} = H).

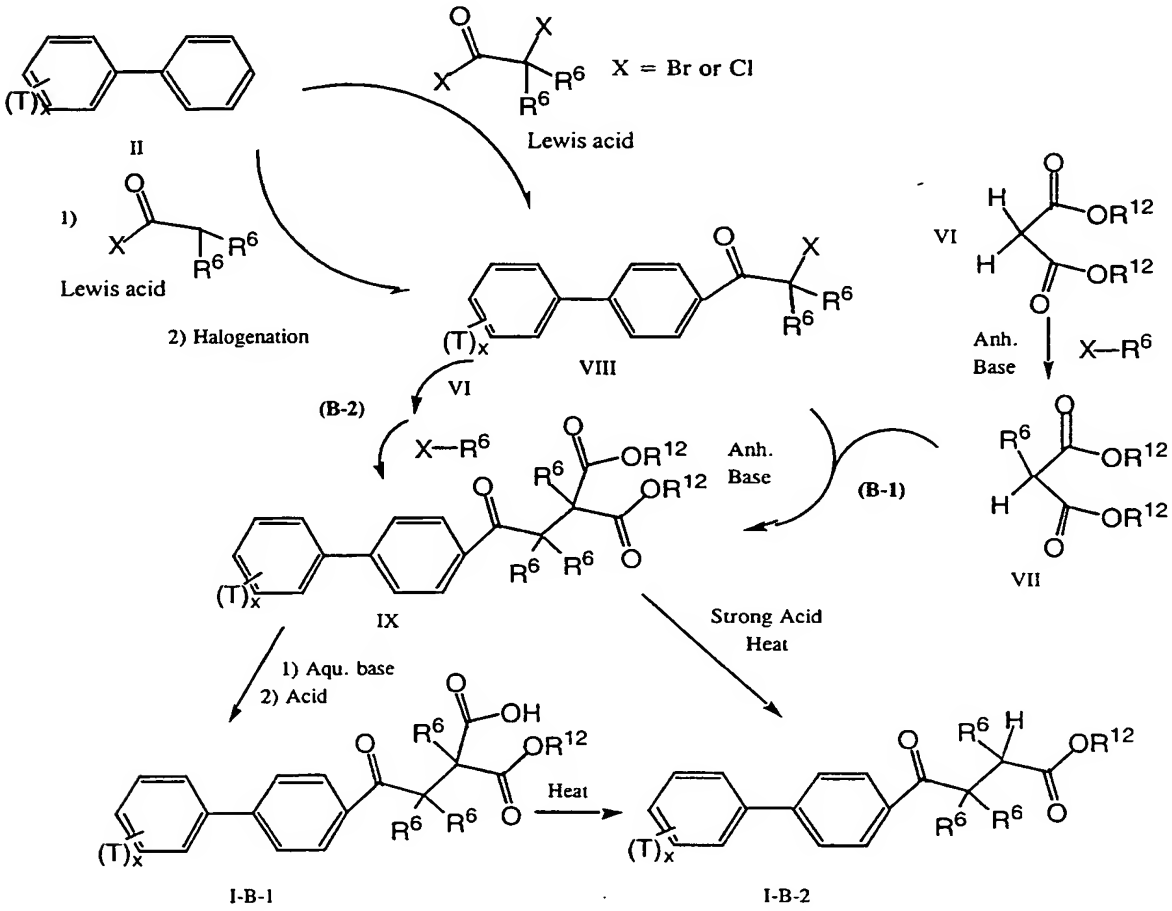
Alternatively, the reaction of VI with VIII can be conducted before that with the alkyl halide to yield the same IX (Method B-2).

Intermediates VIII are formed from biphenyls II in a Friedel-Craft reaction with haloacetyl halides such as bromoacetyl bromide or chloroacetyl chloride. Alternatively, the biphenyl can be reacted with acetyl chloride or acetic anhydride and the

resultant product halogenated with, for example, bromine to yield intermediates VIII (X = Br).

Method B has the advantage of yielding single regio isomers whereas Method A yields mixtures. Method B is especially useful when the side chains R^6 contain aromatic or heteroaromatic rings that may participate in intramolecular acylation reactions to give side products if Method A were to be used. This method is also very useful when the R^6 group adjacent to the carboxyl of the final compound contains heteroatoms such as oxygen, sulfur, or nitrogen, or more complex functions such as imide rings.

Method B



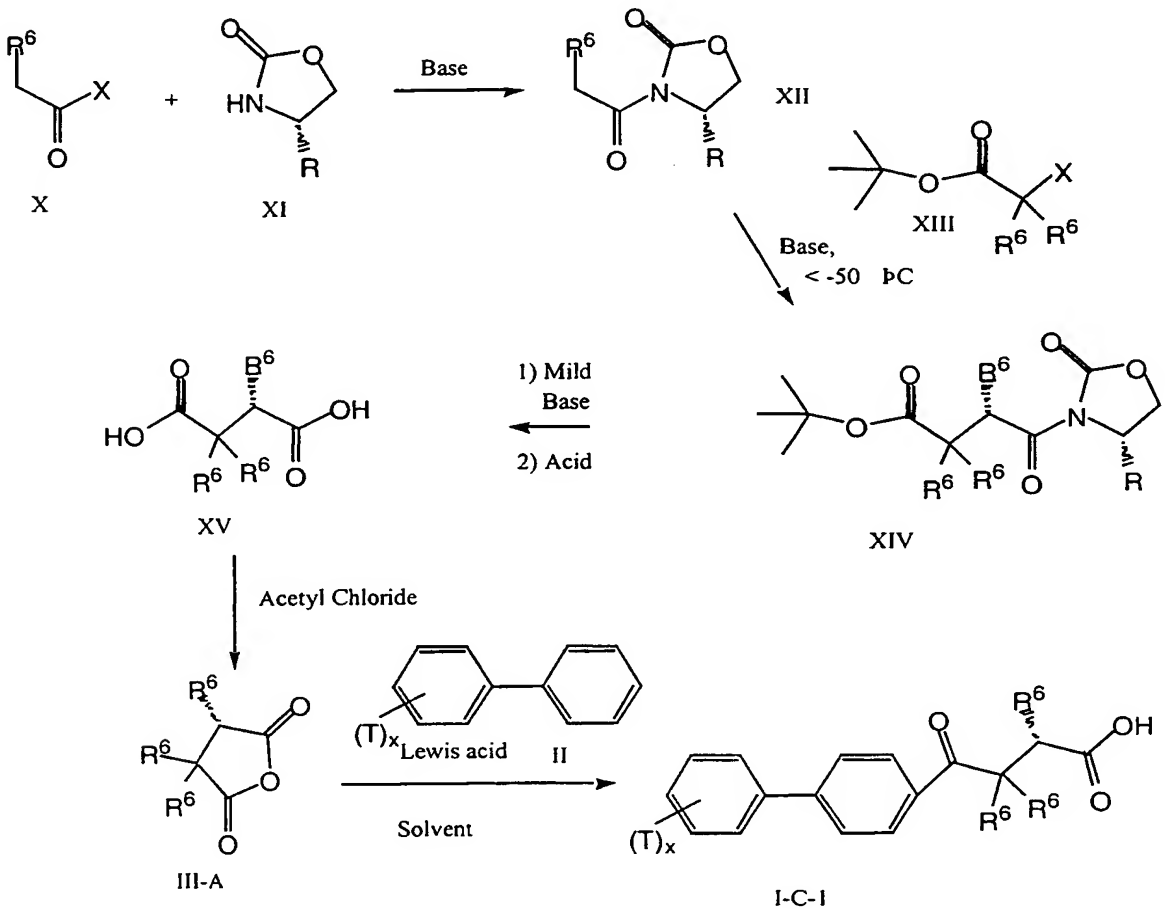
General Meth d C - Especially useful is the use of chiral HPLC to separate the enantiomers of racemates of key intermediates or final products (see, for example, D. Arlt, B. Boemer, R Grosser and W. Lange, *Angew. Chem. Int. Ed. Engl. 30 (1991) No. 12*). The key intermediates are prepared as pure enantiomers by use of a chiral auxiliary route - see, for example: D.A. Evans, Aldrichimica Acta, 15(2), 23 (1982) and other similar references known to one skilled in the art.

C-1. Acid halide X is reacted with the lithium salt of chiral auxiliary XI (R is often isopropyl or benzyl) to yield intermediate XII, which in turn is alkylated at low temperatures (typically under -50°C) with halo-tert-butylacetyl compound XIII to yield pure isomer XIV. The use of opposite chirality XI yields opposite chirality XIV. Conversion of XIV to the enantiomerically pure diacid XV is accomplished by treatment with lithium hydroxide/hydrogen peroxide in THF/water, followed by acids such as trifluoroacetic acid. The compound XV is then converted to enantiomerically pure anhydride III-A by treatment with acetyl chloride. The use of a Friedel-Crafts reaction as in method A then converts III-A to I-C-1.

C-2. Biphenyl starting material II may also first be reacted in a Friedel-Crafts reaction as earlier described with succinic anhydride followed by Fisher esterification with a lower alcohol such as methanol in the presence of a strong acid such as sulfuric acid to form acyl derivative I-C-2. The carbonyl group of this material is then blocked as a ketal such as that formed by treatment with 1,2-bistrimethylsilyloxyethane in the presence of a catalyst such as trimethyl-silyltriflate in a suitable solvent. Many other ketal derivatives and reaction conditions familiar to those skilled in the art can also be used in this step. Basic hydrolysis of the ester followed by reaction of the resultant I-C-3 with XI in the presence of an amide coupling agent such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide yields amide I-C-4. Reaction of this chiral amide with an alkylating agent such as alkyl or arylalkyl triflate or halide yields enantiomerically enriched product I-C-5 which can be converted to ketal acid I-C-6 by treatment with a weak base such as lithium

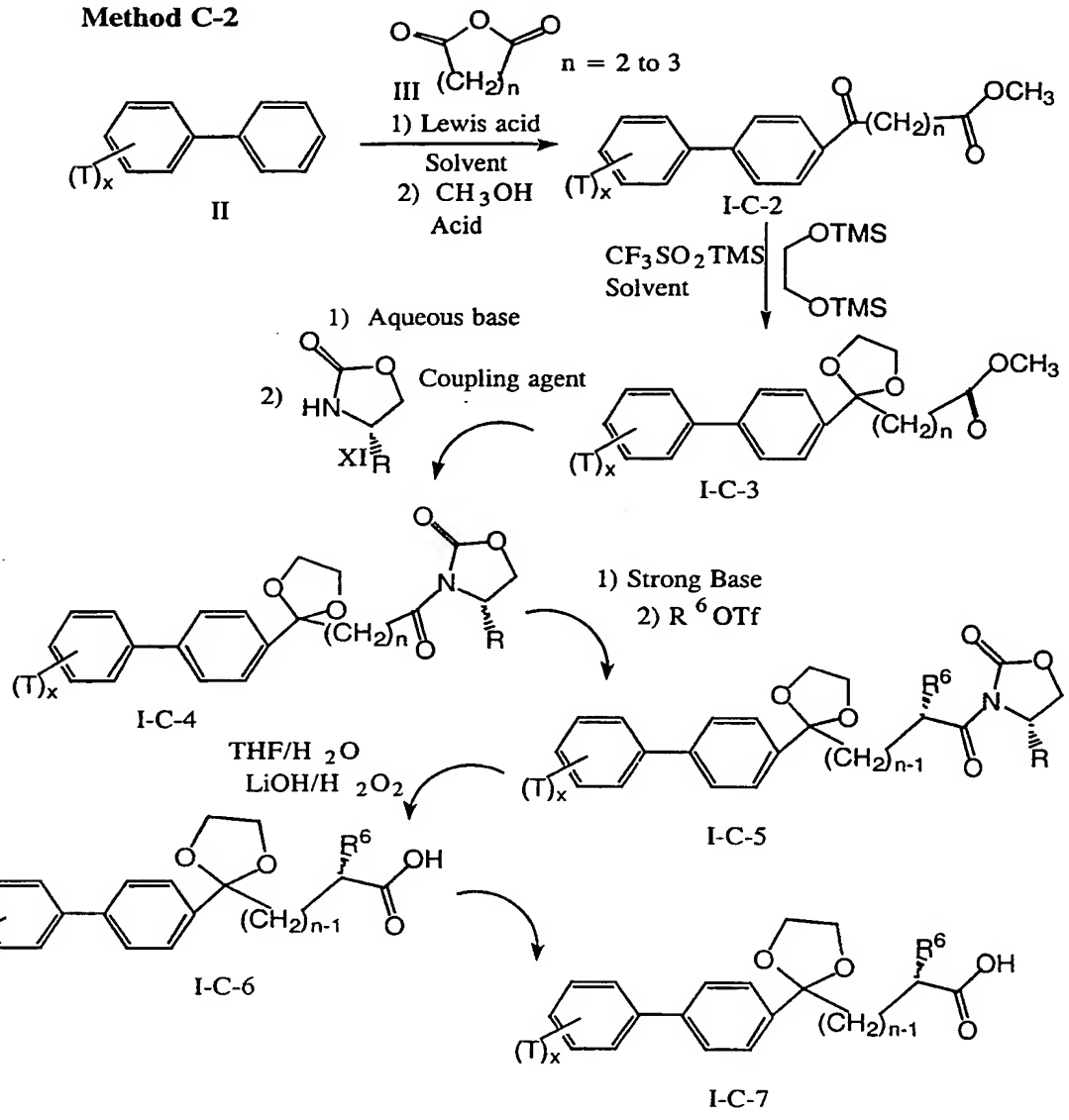
hydroxide/hydrogen peroxide and then to keto acid I-C-7 by treatment with an acid. These deblocking steps can be conducted in either order.

Method C-1



5

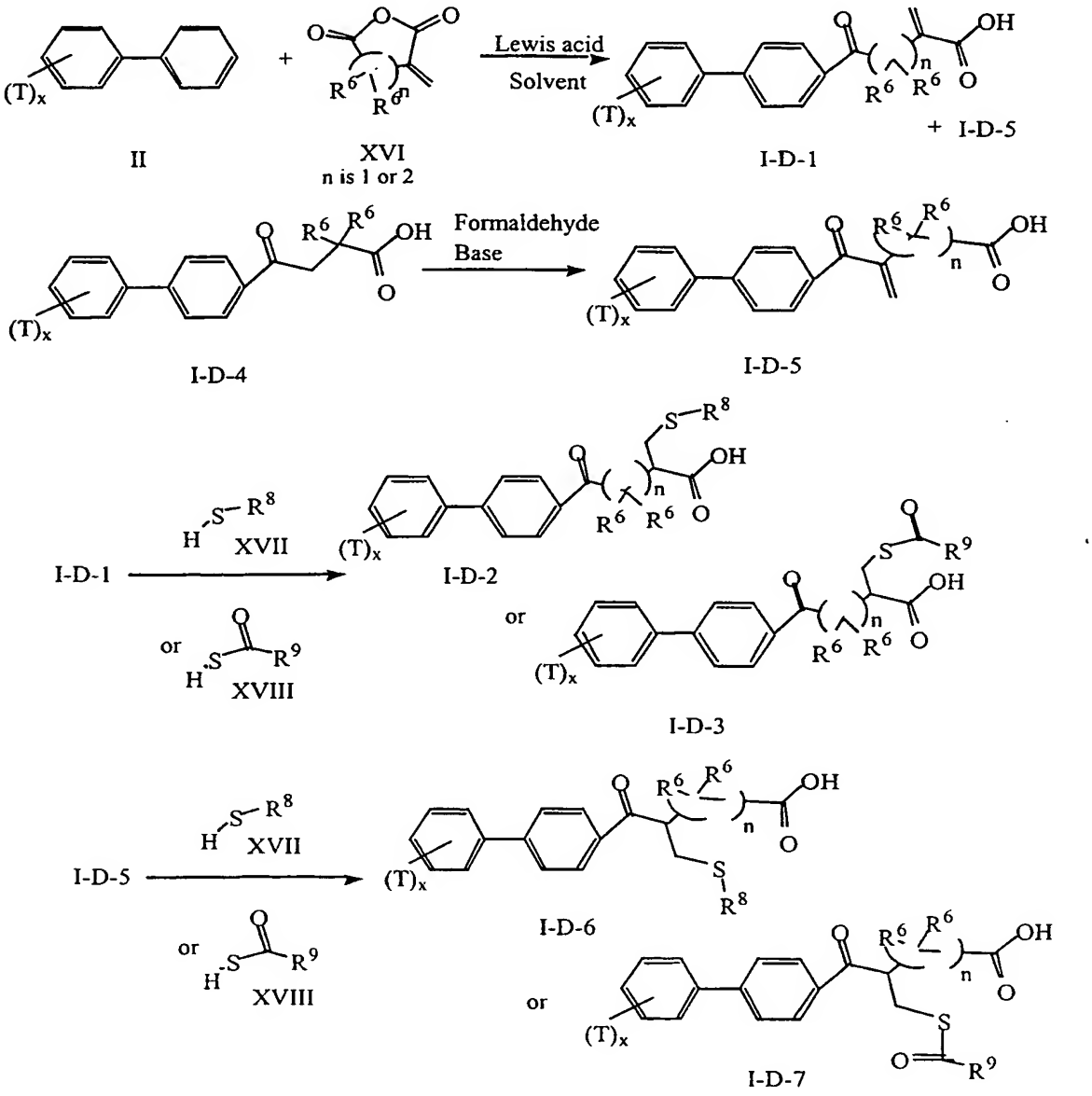
Method C-2



General Method D - Key intermediates in which R^6 are alkyl- or aryl- or heteroaryl- or acyl- or heteroarylcarbonyl-thiomethylene are prepared by methods analogous to those described in the patent publication WO 90/05719. Thus substituted itaconic anhydride XVI (n = 1) is reacted under Friedel-Crafts conditions to yield acid I-D-1 which can be separated by chromatography or crystallization from small amounts of isomeric I-D-5. Alternatively, I-D-5 are obtained by reaction of key intermediates I-D-4 (from any of Methods A through C) with formaldehyde in the presence of a base.

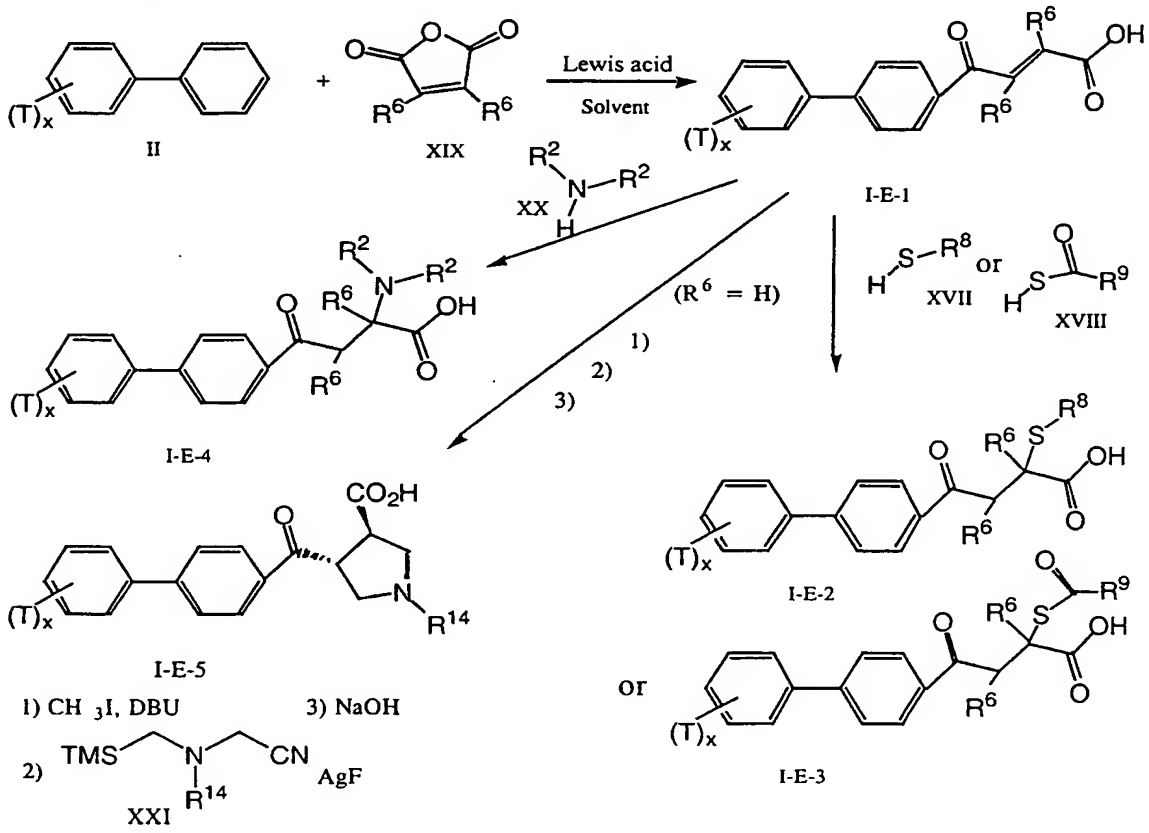
Compounds I-D-1 or I-D-5 are then reacted with a mercapto derivative XVII or XVIII in the presence of a catalyst such as potassium carbonate, ethyldiisobutylamine, tetrabutylammonium fluoride or free radical initiators such as azobisisobutyronitrile (AIBN) in a solvent such as dimethylformamide or tetrahydrofurane to yield key intermediates I-D-2, I-D-3, I-D-6 or I-D-7.

Method D



General Method E - Reaction of optionally substituted maleic anhydride XIX under Friedel-Crafts conditions with II yields key intermediate I-E-1, which in turn is reacted with either of mercapto derivatives XVII or XVIII to yield key intermediates I-E-2 or I-E-3, or with substituted amine XX to yield key intermediate I-E-4. Esterification of I-E-1 (R6 = H) with CH_3I/DBU followed by reagent XXI and AgF and then basic hydrolysis yields pyrrolidine key intermediate I-E-5. R^{14} can be various alkyl or arylalkyl groups including benzyl. Reaction of the intermediate ester (from step 2) with benzyloxycarbonyl chloride in THF at reflux followed by hydrolysis yields key intermediates in which R^{14} is benzyloxycarbonyl.

Method E



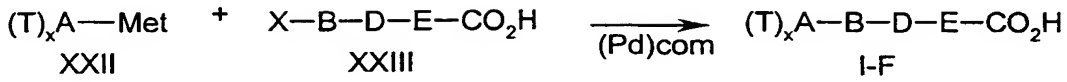
General Method F - Biaryl key intermediates such as those of this application may also be prepared by Suzuki or Stille cross-coupling reactions of aryl or heteroaryl metallic compounds in which the metal is zinc, tin, magnesium, lithium, boron, silicon, copper, cadmium or the like with an aryl or heteroaryl halide or triflate (trifluo-

romethane-sulfonate) or the like. In the equation below either Met or X is the metal and the other is the halide or triflate. Pd(com) is a soluble complex of palladium such as tetrakis(triphenylphosphine)-palladium(0) or bis-(triphenylphos-phine)-palladium(II) chloride. These methods are well known to those skilled in the art. See, for example, A. Suzuki, Pure Appl. Chem., 66, 213 - 222 (1994); A. Suzuki, Pure Appl. Chem., 63, 419 - 422 (1991); and V. Farina and G. Roth, "Metal-Organic Chemistry" Volume 5 (Chapter 1), 1994.

The starting materials XXIII (B = 1,4-phenylene) are readily formed using methods analogous to those of methods A, B or C but using a halobenzene rather than a biphenyl as starting material. When desired, the materials in which X is halo can be converted to those in which X is metal by reactions well known to those skilled in the art such as treatment of a bromo intermediate with hexamethylditin and palladium tetrakistriphenylphosphine in toluene at reflux to yield the trimethyltin intermediate. The starting materials XXIII (B = heteroaryl) are most conveniently prepared by method C but using readily available heteroaryl rather than biphenyl starting materials. The intermediates XXII are either commercial or easily prepared from commercial materials by methods well known to those skilled in the art.

These general methods are useful for the preparation of key intermediates for which Friedel-Crafts reactions such as those of Methods A, B, C, D or E would lead to mixtures with various biaryl acylation patterns. Method F is also especially useful for the preparation of key intermediates in which the aryl groups A or B contain one or more heteroatoms (heteroaryls) such as those compounds that contain thiophene, furan, pyridine, pyrrole, oxazole, thiazole, pyrimidine or pyrazine rings or the like instead of phenyls.

Method F



T, x, A, B, E and D as in Structure I

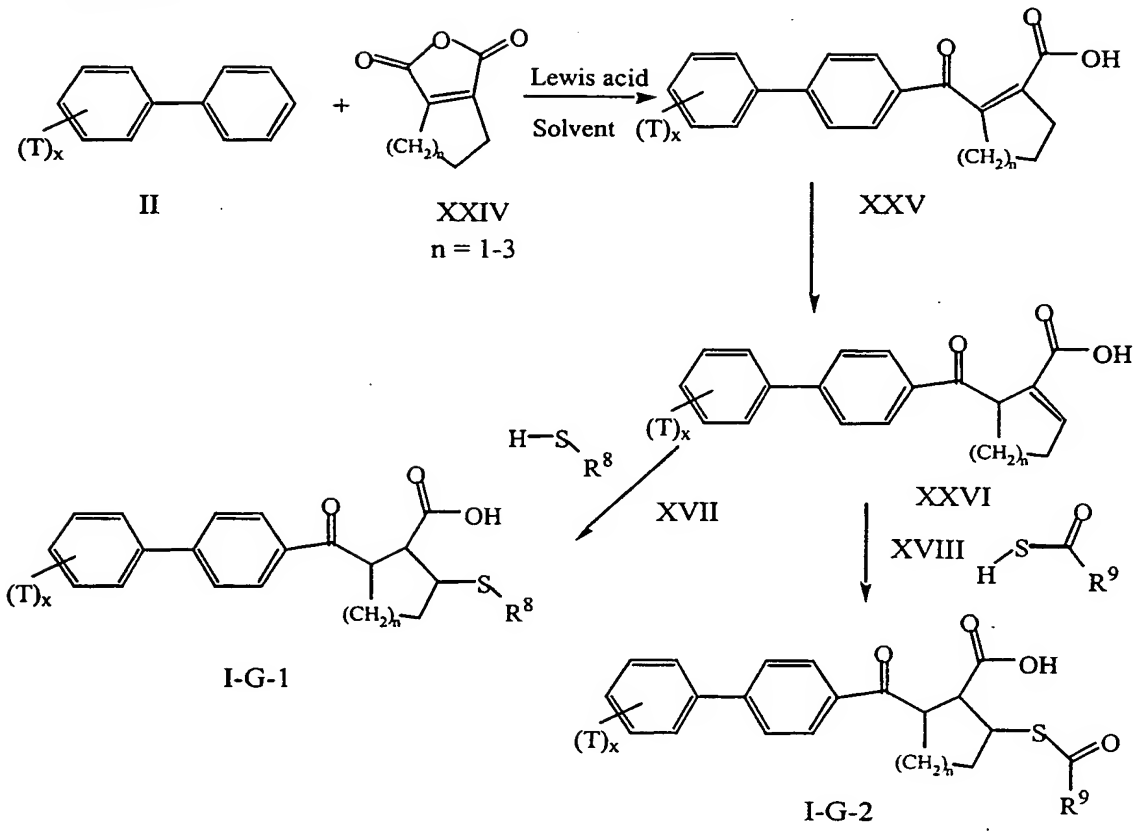
Met = Metal and X = Halide or Triflate

or

Met = Halide or Triflate and X = Metal

General Method G - When the R^6 groups of method F form together a 4 - 7 membered carbocyclic ring as in Intermediate XXV below, the double bond can be moved out of conjugation with the ketone group by treatment with two equivalents of a strong base such as lithium diisopropylamide or lithium hexamethylsilylamide or the like followed by acid quench to yield compounds with the structure XXVI. Reaction of XXVI with mercapto derivatives using methods analogous to those of General Method D then leads to key intermediate I-G-1 or I-G-2.

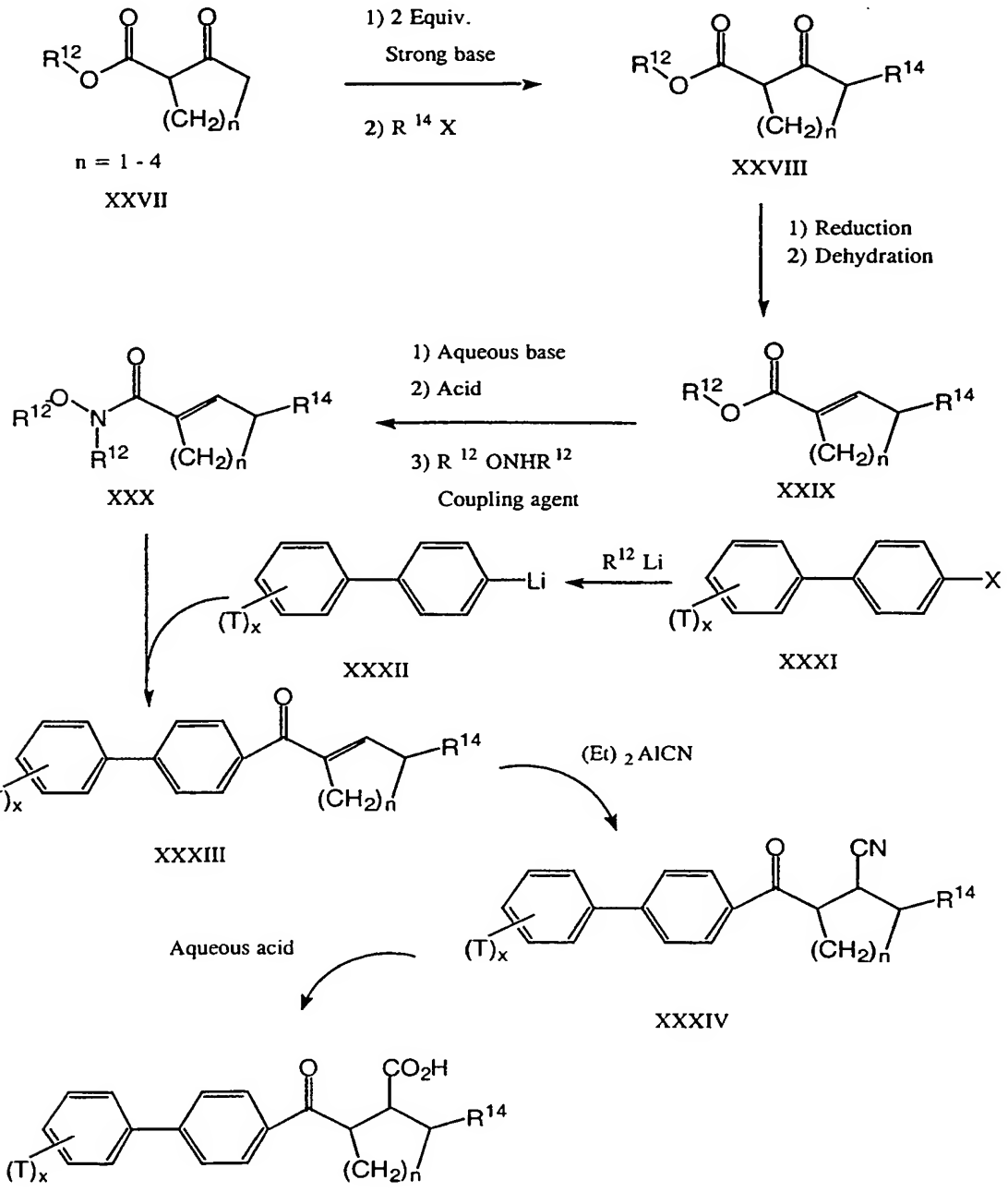
Method G



General Method H - Key intermediates in which two R^6 groups form a 4 - 7 member carbocyclic ring as in I-H below and R^{14} is alkyl or arylalkyl are prepared according to method H. Starting material XXVII is reacted with two equivalents of a strong base such as lithium diisopropylamide (LDA) followed by an alkyl or aryl-alkyl halide ($R^{14}X$) to yield intermediate XXVIII. This material is then reduced to the alcohol with a reducing agent capable of selective reduction of the ketone such as sodium borohydride, followed by dehydration with triphenylphosphine / diethyl azo-dicarboxylate (DEAD) in a suitable solvent such as THF at reflux to yield XXIX. Hydrolysis of the ester with aqueous base followed by amide formation with $R^{12}ONHR^{12}$ (R is (C_1-C_4)-alkyl, but usually CH_3) in the presence of a coupling agent such as dicyclohexyldiimide (DCC) yields XXX. Other acyl activating groups well known to those skilled in the art such as acid chlorides or mixed anhydrides could be used instead of XXX. Substituted biphenyl halide XXXI is reacted with an alkyl lithium such as two equivalents of t-butyl lithium to yield lithiated biphenyl

XXXII which is then reacted with activated acyl compound XXX. The resultant intermediate XXXIII is then treated with diethylaluminum cyanide to yield intermediate XXXIV which is then hydrolyzed with aqueous acid to yield key intermediate I-H which is purified by chromatography on silica gel to afford pure isomers.

Method H

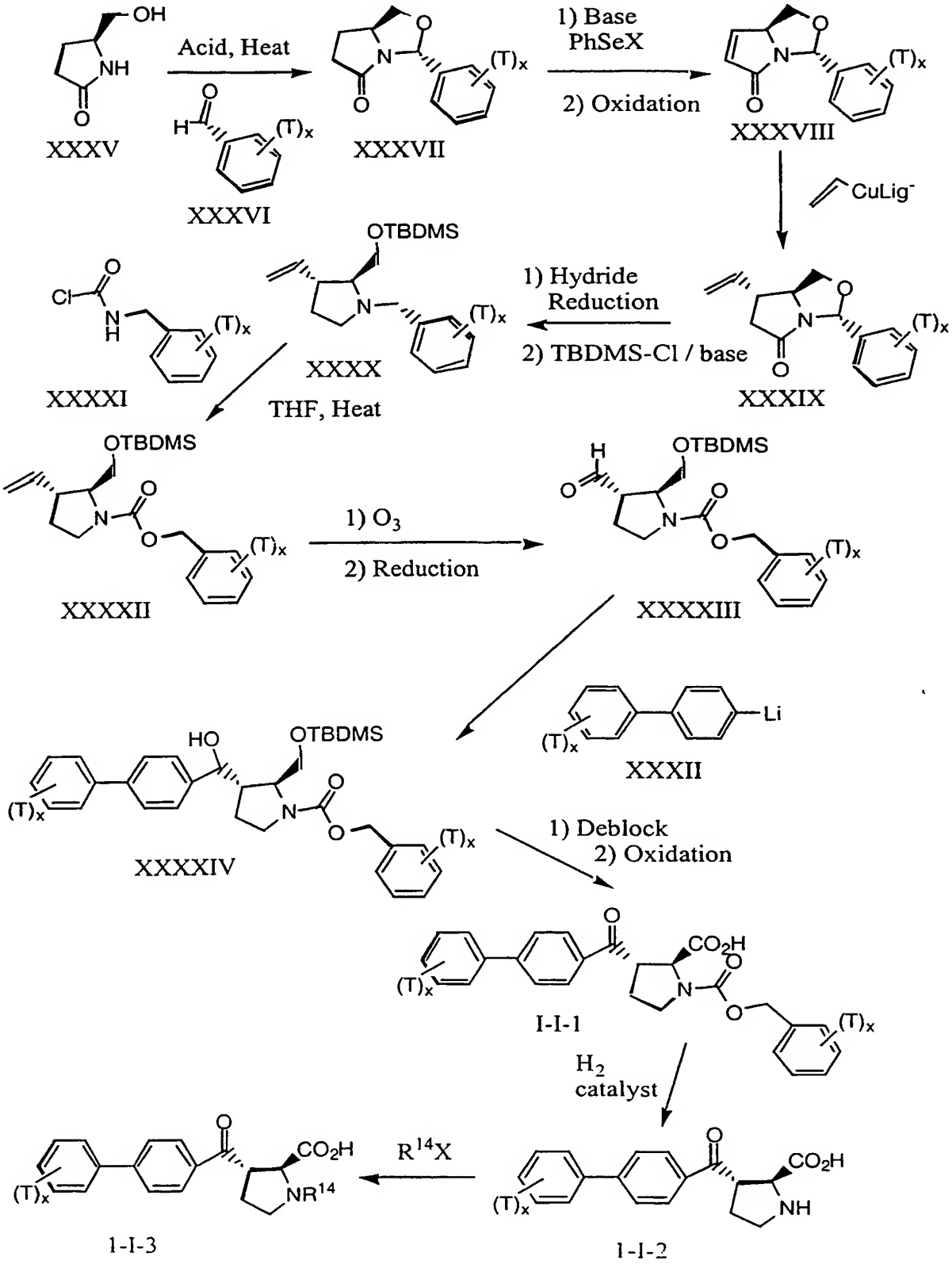


General Method I - Key intermediates in which two R6 groups together form a pyrrolidine ring are prepared according to method I. Starting material XXXV (L-pyroglutaminol) is reacted under acid catalysis with benzaldehyde XXXVI (may be substituted) to yield bicyclic derivative XXXVII. A double bond is then introduced using phenylselenenyl methodology well known to those skilled in the art to yield XXXVIII, which, in turn, is reacted with a vinylcopper (I) complex to yield conjugate addition product XXXIX. Such reactions in which Lig can be, for example, another equivalent of vinyl group or halide are well known to those skilled in the art. Hydride reduction (lithium aluminum hydride or the like) of XXXIX followed by standard blocking with, for example, t-butyldimethylsilylchloride yields XXXX which in turn is reacted with an optionally substituted benzylchloroformate XXXXI to yield XXXXII. Ozonolysis of this intermediate followed by reductive workup (dimethylsulfide, zinc/acetic acid or the like) leads to aldehyde XXXXIII. Reaction of this aldehyde with a biphenyl organometallic such as XXXII yields alcohol XXXXIV. Deblocking of the silyl group with, for example, tetrabutyl-ammonium fluoride followed by oxidation with, for example, pyridiniumdichromate or the like yields key intermediate 1-I-1 in which R^{14} is a carbobenzyloxy group.

Alternatively the carbobenzyloxy group is removed by reaction with hydrogen and a catalyst such as palladium on carbon to yield the unsubstituted key intermediate 1-I-2 optionally followed by N-alkylation to yield key intermediate 1-I-3. These final steps are well known to those skilled in the art. Alternatively the intermediate XXXX can be directly treated with ozone followed by the other steps of this method to yield 1-I-3, in which R^{14} is optionally substituted benzyl rather than as in 1-I-1.

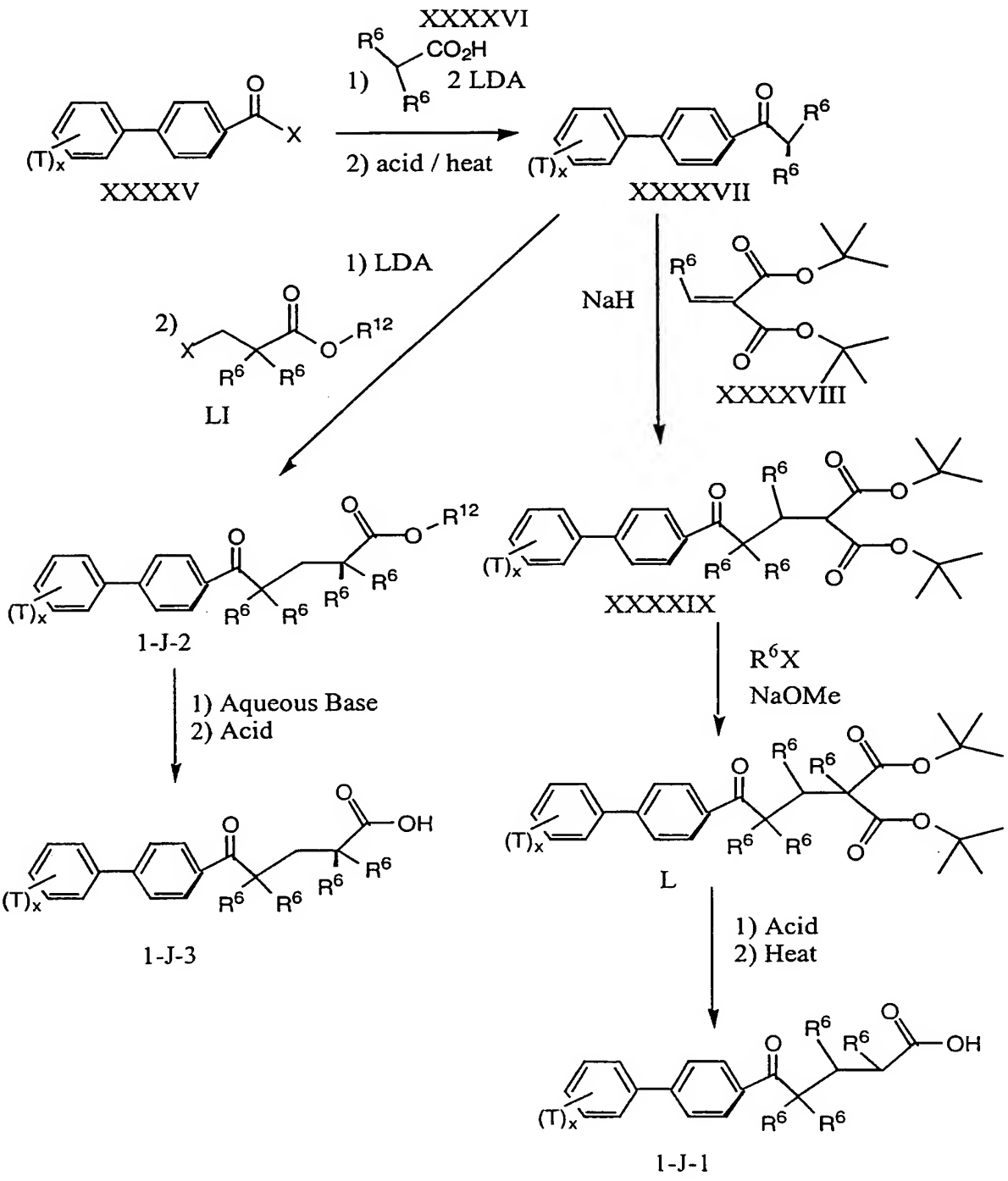
This method is especially useful to prepare single enantiomers because starting material XXXV is available as either the isomer as drawn or as D-pyroglutaminol to yield enantiomeric products.

Meth d I


OH
NH
O
XXXV
Acid, Heat
H
O
(T)x
XXXVI
O
N
(T)x
O
XXXVII
1) Base
PhSeX
2) Oxidation
XXXVIII
CuLig⁻
1) Hydride
Reduction
2) TBDMS-Cl / base
XXXIX
OTBDMS
XXXX
Cl
N
H
XXXXI
THF, Heat
1) O3
2) Reduction
XXXXII
XXXXIII
HO
XXXXIV
Li
XXXII
1) Deblock
2) Oxidation
CO2H
I-I-1
H2
catalyst
R14X
NR14
1-I-3
1-I-2

General Method J - The key intermediates in which E represents a substituted chain of 3 carbons are prepared by method J. Intermediates XXXXVII, if not available from commercial sources, are prepared by reaction of an activated biphenylcarboxylic acid derivative XXXXV with substituted acetic acid XXXXVI which has been converted to its bis-anion with two equivalents of a strong base such as LDA followed by heating to decarboxylate the intermediate keto acid. Product XXXXVII is then treated with methylenemalonate derivative XXXXVIII in the presence of a strong base such as sodium hydride to yield substituted malonate XXXXIX. This malonate can be further alkylated under conditions familiar to those skilled in the art to yield L which in turn is treated with acid and then heated to yield key intermediate 1-J-1. Alternatively the final alkylation can be omitted to yield products in which the R^6 adjacent to the carboxyl is H. Alternatively XXXXVII can be alkylated with 3-halopropionate ester LI in the presence of base such as LDA to yield ester 1-J-2 which can then be hydrolyzed with aqueous base to yield key intermediate 1-J-3 upon treatment with acid. This method is especially useful if any of the groups R^6 contain aromatic residues.

Method J



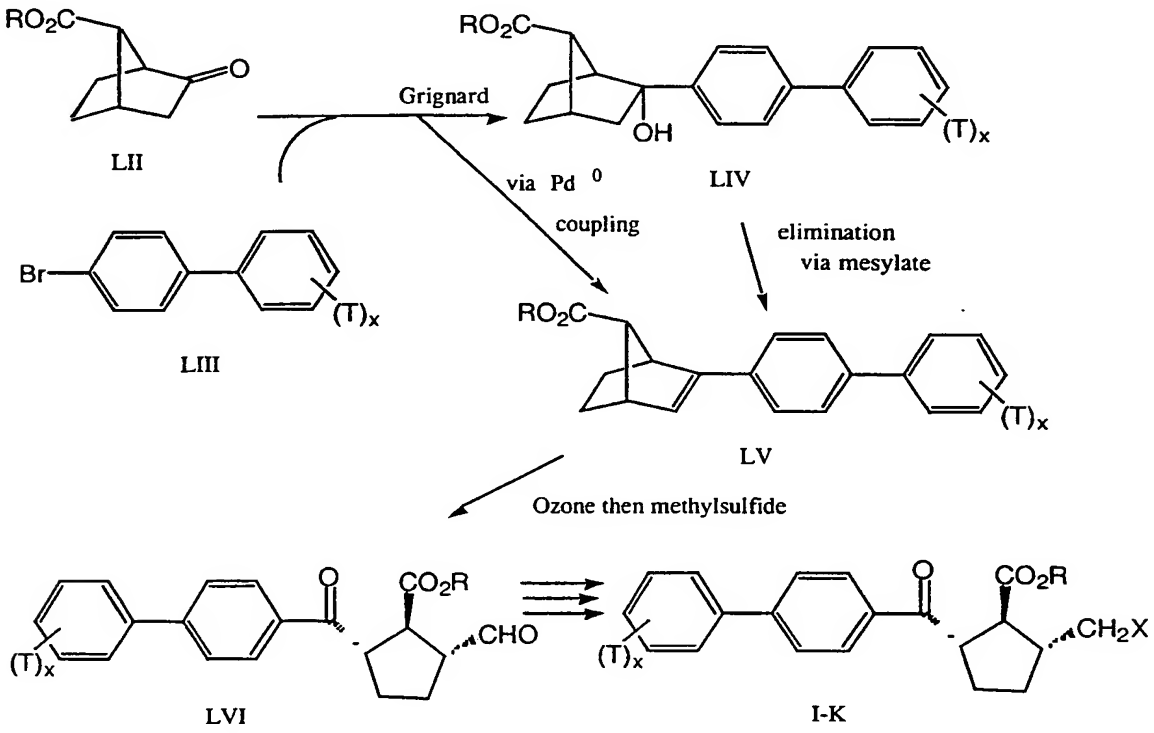
Method K - The key intermediates in which two R^6 groups are joined to form a substituted 5-member ring are most conveniently prepared by method K. In this method

acid LII (R = H) is prepared using the protocols described in *Tetrahedron*, Vol. 37, Suppl., **1981**, 411. The acid is protected as an ester (R = benzyl or 2-(trimethylsilyl)ethyl) by use of coupling agents such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and procedures well known to those skilled in the art. Substituted bromobiphenyl LIII is converted to its Grignard reagent by treatment with magnesium which is then reacted with LII to yield alcohol LIV. Alcohol LIV is eliminated via base treatment of its mesylate by using conditions well known to those skilled in the art to yield olefin LV. Alternatively LIII is converted to a trimethyltin intermediate via initial metallation of the bromide with n-butyllithium at low temperature (-78°C) followed by treatment with chlorotrimethyltin and LII is converted to an enoltriflate by reaction with 2-[N,N-bis(trifluoromethylsulfonyl)-amino]-5-chloropyridine in the presence of a strong aprotic base. The tin and enoltriflate intermediates are then coupled in the presence of a Pd^0 catalyst, CuI and $AsPh_3$ to yield directly intermediate LV. Ozonolysis of LV (workup with methyl sulfide) yields aldehyde LVI. Alternatively treatment with OsO_4 followed by HIO_4 converts LV to LVI.

Conversion of intermediate LVI to key intermediate I-K is accomplished in several ways depending on the identity of side chain function X. Reaction of LVI with Wittig reagents followed by hydrogenation yields products in which X is alkyl, aryl or arylalkyl. Reduction of aldehyde LVI with LAH yields alcohol I-K (X = OH). The alcohol is converted to phenyl ethers or N-phthalimidoyl compounds by use of the appropriate starting materials and Mitsunobu conditions well known to those skilled in the art; see O. Mitsunobu, Synthesis, 1 (1981). Alternatively the alcohol of I-K (X = OH) is converted to a leaving group such as tosylate (X = OTs) or bromide (X = Br) by conditions well known to those skilled in the art and then the leaving group is displaced by sulfur or azide nucleophiles to yield products with X = thioether or azide which in turn is reduced and acylated to yield amides (X = NHAcyl). Direct acylation of the alcohol I-K (X = OH) yields key intermediates in which X = OAcyl and reaction of the alcohol with various alkyl halides in the presence of base yields alkyl ethers ($X = OR^2$). In each case a final step is removal of acid blocking group R

to yield acids (R = H) by using conditions which depend on the stability of R and X, but in all cases well known to those skilled in the art such as removal of benzyl by base hydrolysis or of 2-(trimethylsilyl)ethyl by treatment with tetrabutylammonium fluoride.

Method K



Suitable pharmaceutically acceptable salts of the compounds of the present invention that contain an acidic moiety include addition salts formed with organic or inorganic bases. The salt forming ion derived from such bases can be metal ions, e.g., aluminum, alkali metal ions, such as sodium of potassium, alkaline earth metal ions such as calcium or magnesium, or an amine salt ion, of which a number are known for this purpose. Examples include ammonium salts, arylalkylamines such as dibenzylamine and *N,N*-dibenzylethylenediamine, lower alkylamines such as methylamine, *t*-butylamine, procaine, lower alkylpiperidines such as *N*-ethylpiperidine, cycloalkylamines such as cyclohexylamine or dicyclohexylamine, 1-adamantylamine,

benzathine, or salts derived from amino acids like arginine, lysine or the like. The physiologically acceptable salts such as the sodium or potassium salts and the amino acid salts can be used medicinally as described below and are preferred.

Suitable pharmaceutically acceptable salts of the compounds of the present invention that contain a basic moiety include addition salts formed with organic or inorganic acids. The salt forming ion derived from such acids can be halide ions or ions of natural or unnatural carboxylic or sulfonic acids, of which a number are known for this purpose. Examples include chlorides, acetates, tartrates, or salts derived from amino acids like glycine or the like. The physiologically acceptable salts such as the chloride salts and the amino acid salts can be used medicinally as described below and are preferred.

These and other salts which are not necessarily physiologically acceptable are useful in isolating or purifying a product acceptable for the purposes described below.

The salts are produced by reacting the acid form of the invention compound with an equivalent of the base supplying the desired basic ion or the basic form of the invention compound with an equivalent of the acid supplying the desired acid ion in a medium in which the salt precipitates or in aqueous medium and then lyophilizing. The free acid or basic form of the invention compounds can be obtained from the salt by conventional neutralization techniques, e.g., with potassium bisulfate, hydrochloric acid, sodium hydroxide, sodium bicarbonate, etc.

The compounds of the present invention are expected to inhibit the matrix metalloproteases MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-12, MMP-13, and the related protease TACE, as well as the release of TNFα in vivo, and are therefore expected to be useful for treating or preventing the conditions referred to in the background section. As other MMPs not listed above share a high degree of homology with those listed above, especially in the catalytic site, it is deemed that compounds of the invention should also inhibit such other MMPs to varying degrees. Varying

the substituents on the biaryl portions of the molecules, as well as those of the R^6 groups of the claimed compounds, is expected to affect the relative inhibition of the listed MMPs. Thus compounds of this general class can be "tuned" by selecting specific substituents such that inhibition of specific MMP(s) associated with specific pathological conditions can be enhanced while leaving non-involved MMPs less affected.

The method of treating matrix metalloprotease-mediated or TNFα release-mediated conditions may be practiced in mammals, including humans, which exhibit such conditions.

The inhibitors of the present invention are contemplated for use in veterinary and human applications. For such purposes, they will be employed in pharmaceutical compositions containing active ingredient(s) plus one or more pharmaceutically acceptable carriers, diluents, fillers, binders, and other excipients, depending on the administration mode and dosage form contemplated.

Administration of the inhibitors may be by any suitable mode known to those skilled in the art. Examples of suitable parenteral administration include intravenous, intraarticular, subcutaneous and intramuscular routes. Intravenous administration can be used to obtain acute regulation of peak plasma concentrations of the drug. Improved half-life and targeting of the drug to the joint cavities may be aided by entrapment of the drug in liposomes. It may be possible to improve the selectivity of liposomal targeting to the joint cavities by incorporation of ligands into the outside of the liposomes that bind to synovial-specific macromolecules. Alternatively intramuscular, intraarticular or subcutaneous depot injection with or without encapsulation of the drug into degradable microspheres e.g., comprising poly(DL-lactide-co-glycolide) may be used to obtain prolonged sustained drug release. For improved convenience of the dosage form it may be possible to use an i.p. implanted reservoir and septum such as the Percuseal system available from Pharmacia. Improved convenience and patient compliance may also be achieved by the use of either injector pens (e.g. the

Novo Pin or Q-pen) or needle-free jet injectors (e.g. from Bioject, Mediject or Becton Dickinson). Prolonged zero-order or other precisely controlled release such as pulsatile release can also be achieved as needed using implantable pumps with delivery of the drug through a cannula into the synovial spaces. Examples include the subcutaneously implanted osmotic pumps available from ALZA, such as the ALZET osmotic pump.

Nasal delivery may be achieved by incorporation of the drug into bioadhesive particulate carriers (<200 μm) such as those comprising cellulose, polyacrylate or polycarbophil, in conjunction with suitable absorption enhancers such as phospholipids or acylcarnitines. Available systems include those developed by DanBiosys and Scios Nova.

Oral delivery may be achieved by incorporation of the drug into tablets, coated tablets, dragées, hard and soft gelatine capsules, solutions, emulsions or suspensions. Oral delivery may also be achieved by incorporation of the drug into enteric coated capsules designed to release the drug into the colon where digestive protease activity is low. Examples include the OROS-CT/Osmet™ and PULSINCAP™ systems from ALZA and Scherer Drug Delivery Systems respectively. Other systems use azo-crosslinked polymers that are degraded by colon specific bacterial azoreductases, or pH sensitive polyacrylate polymers that are activated by the rise in pH at the colon. The above systems may be used in conjunction with a wide range of available absorption enhancers.

Rectal delivery may be achieved by incorporation of the drug into suppositories.

The compounds of this invention can be manufactured into the above listed formulations by the addition of various therapeutically inert, inorganic or organic carriers well known to those skilled in the art. Examples of these include, but are not limited to, lactose, corn starch or derivatives thereof, talc, vegetable oils, waxes, fats, polyols such as polyethylene glycol, water, saccharose, alcohols, glycerin and the like.

Various preservatives, emulsifiers, dispersants, flavorants, wetting agents, anti-oxidants, sweeteners, colorants, stabilizers, salts, buffers and the like are also added, as required to assist in the stabilization of the formulation or to assist in increasing bioavailability of the active ingredient(s) or to yield a formulation of acceptable flavor or odor in the case of oral dosing.

The amount of the pharmaceutical composition to be employed will depend on the recipient and the condition being treated. The requisite amount may be determined without undue experimentation by protocols known to those skilled in the art. Alternatively, the requisite amount may be calculated, based on a determination of the amount of target enzyme which must be inhibited in order to treat the condition. It is expected that the compounds of the invention generally will be administered in doses in the range of 0.01-100 mg per kg of body weight per day.

The matrix metalloprotease inhibitors of the invention are useful not only for treatment of the physiological conditions discussed above, but are also useful in such activities as purification of metalloproteases and testing for matrix metalloprotease activity. Such activity testing can be both *in vitro* using natural or synthetic enzyme preparations or in vivo using, for example, animal models in which abnormal destructive enzyme levels are found spontaneously (use of genetically mutated or transgenic animals) or are induced by administration of exogenous agents or by surgery which disrupts joint stability.

Biological Protocols

Inhibitory activities of the compounds of the invention against matrix metalloproteases and production of TNFα may be determined as described below.

Preparation of Gelatinase-B (MMP-9):

MMP-9 is isolated modifying the previously described procedures of Hibbs et al (J. Biol. Chem., 260, 2493-2500, 1984) and Wilhelm et al (J. Biol. Chem., 264, 17213-17221, 1989). Briefly, polymorphonuclear leukocytes (PMN) preparations are isolated as described above from 3 or more units of freshly drawn whole blood. Cells are resuspended in phosphate buffered saline (PBS) containing 100 ng/ml phorbol myristate acetate (PMA) in the presence of 50 mM di-isopropylfluorophospate (DFP), 1 μg/ml leupeptin and aprotinin, and 1 mg/ml catalase for 1 hr at 37°C. Supernatants are collected by centrifugation (300 x g) and the samples are frozen at -70°C. All chromatographic methods are performed at 4°C. Thawed samples are concentrated 5-fold using an Amicon chamber equipped with a YM-10 membrane. The concentrate is pressure dialyzed against 0.02M Tris-HCl, 0.1 M NaCl, 1 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.001% Brij-35, 0.02% sodium azide (NaN_3), pH 7.5 and applied to DEAE ion exchange chromatography resin which is previously equilibrated with the same buffer at a flow rate of 0.4 ml/min. The column is extensively washed with the same buffer and gelatinase is eluted as 4 ml fractions from the column with 0.02M Tris-HCl, 0.5 M NaCl, 1 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.001% Brij-35, 0.02% NaN_3, pH 7.5. Gelatinase containing fractions are observed by gelatin zymography (see below), loaded onto a gelatin agarose affinity resin and washed with the same buffer. Gelatinase activity is eluted at a flow rate of 1 ml/min from the column as 1 ml fractions with 0.02M Tris-HCl, 1 M NaCl, 1 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.001% Brij-35, 0.02% NaN_3, pH 7.5 containing 10% dimethyl sulfoxide (DMSO). The fractions containing gelatinase activity are pooled and dialyzed against 0.005M Tris-HCl, 5mM NaCl, 0.5 mM $CaCl_2$, 0.1 μM $ZnCl_2$, 0.001% Brij-35, pH 7.4. The protein content associated with material is determined

with a micro-BCA assay (Pierce, Rockford, IL), lyophilized and reconstituted to a desired working concentration (100 μg/ml).

Preparation of Gelatinase-A (MMP-2):

Gelatinase A (MMP-2) is prepared using a vaccinia expression system according to the method of R. Fridman, et al., *J. Biol. Chem.*, 267, 15398 (1992).

Preparation of Recombinant Truncated Prostromelysin (MMP-3):

Truncated Prostromelysin-257 is expressed in a soluble form in E.coli as described by Marcy et al., Biochemistry, 30, 6476-6483, 1991. Soluble truncated prostromelysin is purified by a modification of the monoclonal antibody affinity chromatography method described by Housley et al., J. Biol. Chem., 268, 4481-87, 1993.

P218 Quenched fluorescence Assay for MMP-3 Inhibition:

This assay was originally described by Knight et al., FEBS Letters, 296, 263-266, 1992, for a related substrate. The assay is run continuously in a 3.0ml cuvette using a Perkin-Elmer LS 50 B Spectrofluorimeter at 25 °C in a final volume of 2.0 mls. P218 substrate (10mM) in 100% DMSO is diluted to a final concentration of 2.0 micromolar (μM) into assay buffer: 50mM MES, pH 6.5 containing 150mM NaCl, 10mM CaCl2, 0.005% Brij-35, and 1%(v/v) DMSO. Test compounds(10mM) in DMSO are diluted in assay buffer at an initial concentration of 10 to 100 micromolar. These are diluted to a final concentration in the assay from 10 nM to 1 μM depending upon their potency previously determined in primary thiopeptilide assay described above. The reaction is initiated by the addition of recombinant stromelysin (MMP-3) at a final concentration of 1.0 nM. Upon peptide cleavage, the fluorescent MCA group is detected using an excitation wavelength of 328 nanometers and an emission wavelength of 393 nanometers. The assay is linear from 0.2 to 5nM MMP-3 concentration and percent inhibition is calculated as described above for the pri-

mary thiopeptilide assay and IC_{50} values are determined by a linear regression analysis of percent inhibition versus log drug concentration. The peptide sequence of the MCA substrate, hereinafter designated P218, is shown below:

MCA-Pro-Lys-Pro-**Leu-Ala**-Leu-DPA-Ala-Arg-NH_2

P218

For MMP-3, this substrate has a K_m of 16 μM at pH 6.5 and a kcat/K_m value of 56,000$M^{-1}sec^{-1}$.

P218 Quenched Fluorescence Assay for MMP-12 Inhibition:

This assay is adapted from the one described by Knight et al., FEBS Letters, 296, 263-266 (1992) for MMP-3 and a related substrate. - The rate of hydrolysis of the synthetic substrate H-MCA-Pro-Lys-Pro-Leu-Ala-Leu-DPA-Ala-Arg-NH_2 (P218) by human recombinant MMP-12 is monitored fluorometrically, using an excitation wavelength of 340 nm and an emission wavelength of 395 nm, in the presence or absence of the test compounds. The assay is carried out in buffer containing 50 mM HEPES and 10 mM $CaCl_2$ at pH 7.0. The substrate is made up initially in 100% DMSO to a concentration of 1 x 10^{-2} M, then diluted in assay buffer to a final concentration of 20 μM. Test compounds (10 mM in DMSO) are diluted in assay buffer at an initial concentration of 0.3-1000 nM. These are diluted to a final concentration in the assay from 0.03 nM to 100 nM. The reaction is initiated by the addition of substrate at a final concentration of 20 μM. The total assay volume in a 96 well microtitre plate is 150 μl. Cleavage of the substrate between the Leu-Ala residues allows the fluorescence of the MCA group to be detected on a fluorometer (Cytofluor II) following excitation at 340 nm and emission at 395 nm. Change in fluorescence is continually monitored for a 40 min period.

The K_i's are calculated using the method described by Williams and Morrison, Methods in Enzymology, 63, 437-467 (1979) to measure $K_{i\ apparent}$ for tight binding inhibitors, and is summarised as follows:

$[I]_0 / (1-v_i/v_0) = K_{i\ apparent} \times v_i / v_0 + [E]_0$

$[I]_0$ and $[E]_0$ are inhibitor and enzyme concentrations, and v_i / v_0 are reaction velocities with / without inhibitor. $[I]_0$ is equal to IC_{50} when v_i is half v_0, so that:

$IC_{50} = 0.5 \times [E]_0 + K_{i\ apparent}$

IC_{50}'s are determined at each enzyme concentration (0.044-0.98 µg/ml) using Xlfit software. $K_{i\ apparent}$ is then determined graphically from the plot of IC_{50} versus MMP-12 concentration, using the intercepts to estimate $K_{i\ apparent}$. Thus, intercept values at $IC_{50} = 0$ and $[E]_0 = 0$ are equal to $-2 \times K_{i\ apparent}$ and $K_{i\ apparent}$, respectively. IC_{50} values are calculated using % inhibition values at each enzyme concentration, ensuring data is taken from the linear part of the reaction rate curves. The K_i can then be calculated from the equation:

$K_i = K_{i\ apparent} / (1+S)/K_m$

where S = substrate concentration (20 µM) and K_m = dissociation constant (5.4 µM).

Automated MMP Profiling Assay

This assay is run with a protocol analogous to that reported for MMP-3 inhibition using the synthetic peptide P218 and each of the three enzymes and measuring quenched fluorescence. This assay can be run with each invention compound with the three enzymes in parallel as adapted for a 96-well microtitre plate using a Hamilton AT ® workstation.

The following examples illustrate the selectivity of compounds of the invention for specific human MMPs:

Example	Potency [K_i, nM] vs. MMP-x							
	1	2	3	7	8	9	12	13
4	639	0.5	3.7	>3000	7.5	0.7	0.3	5.6
2	>3000	3.2*	60*	38% inh. at 30 nM	21.6*	1.2	0.03	70*

* IC_{50} [nM]

LPS Induced TNFα Production in Mice

The *in vivo* inhibitory properties of selected compounds can be determined using a murine LPS induced TNFα production *in vivo* model. BALB/c mice (Charles River Breeding Laboratories; Kingston, NY) in groups of ten are treated with either vehicle or compound. After one hour, endotoxin (E. coli lipopolysaccharide (LPS) 100 mg) is administered intraperitoneally (i.p.). After 90 min, animals are euthanized by carbon dioxide asphyxiation and plasma is obtained from individual animals by cardiac puncture into heparinized tubes. The samples are clarified by centrifugation at 12,500 x g for 5 min at 4 °C. The supernatants are decanted to new tubes, which are stored as needed at -20 °C. TNFα levels in sera are measured using a commercial murine TNF ELISA kit (Genzyme).

Other embodiments of the invention will be apparent to the skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

Inhibition of Acute EAE Induction in Mice

Acute EAE (Experimental Autoimmune Encephalomyelitis) is induced by immunization of female SJL-mice with an encephalitic fragment of the myelin constituent proteolipid protein ($PLP_{139-151}$) dissolved in Freunds Complete Adjuvant and additional treatment (i.v.) with *Bordetella pertussis*. EAE-diseased mice develop clinical signs around 10 days after immunization which are accompanied by rapid loss of body weight and which resolve between day 16 and 20. Histopathologically this acute disease is characterized by CNS perivascular and parenchymal inflammation and, if at all, minimal demyelination. Infiltrates are mostly composed of T-cells and macrophages.

Animals (n=5) were treated with Example 4. The compound was administered intraperitonealy twice daily for 20 days at 1, 3, and 10 mg/kg and treatment started at the day of disease induction. The compound was dissolved in Labrafil M 1944CS and the injection volume was 100 µl.

The compound dose-dependently inhibited EAE induction (Figure 1). This experiment clearly indicates the therapeutic potential of the compounds of this invention for the treatment of MS.

Inhibition of Acute EAE Induction in Rats

Acute EAE is induced in female Lewis rats by inoculation of a spinal cord-adjuvant emulsion into each hind paw of the animals. Animals show signs of disease (body weight loss, neurological deficits) starting around day 10 after immunization and resolving between days 17-23.

Animals are treated by per os (p.o.) administration of the test compound. Treatment starts on day 6 post inoculation and stops on day 17. The compound is administered

at 3 different doses (e.g. 1, 10, 30 mg/kg) once daily. Body weight and appearance of neurological signs are checked on a daily basis.

Inhibition of Chronic EAE Induction in Mice

Chronic relapsing EAE is induced by adoptive transfer of $PLP_{139-151}$-responsive lymph node cells to naive female SJL/J recipient mice by injection into the tail vein. 6-8 days later, the mice develop paralysis and incontinence, acute symptoms which correspond to a period of intense inflammation, demyelination and some Wallerian degeneration in the central nervous system. The acute phase peaks from 14-20 days post transfer (dpt) of lymph node cells is followed by remission. The chronic phase of EAE, characterized by spontaneous relapses with severe clinical symptoms, occurs after 21 dpt.

The test compound is administered daily by p.o. application at 3 different doses (e.g. 1, 10, 30 mg/kg). Treatment starts on day 21 post transfer and stops on day 78. Neurological impairment in EAE mice is evaluated every 1-4 days after disease induction. The clinical scores of each individual animal are used to score relapses and to calculate relapse frequency and mean clinical score.

Abbreviations

DMF	dimethylformamide
RT	room temperature
THF	tetrahydrofuran
TBME	tertiary butyl methyl ether

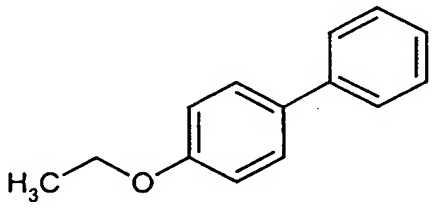
Preparation Examples

Example 1

(rac)-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(4'-ethoxy[1,1'-biphenyl]-4-yl)-4-oxobutanoic acid

Intermediate 1A

4-Ethoxy-1,1'-biphenyl



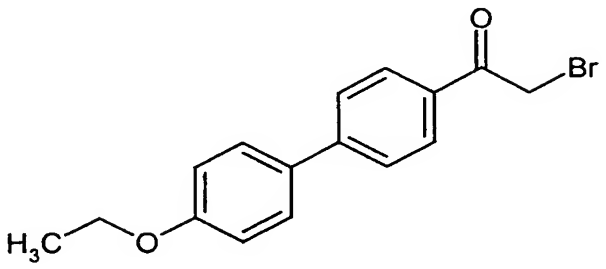
Iodoethane (68.7 g, 35,57 mL, 440.6 mmol) was added to a suspension of 50 g (170.2 mmol) of 4-hydroxy-1,1'-biphenyl and 40.6 g (293.75 mmol) K_2CO_3 in 60 mL acetone. The resulting reaction mixture was stirred under reflux for 16 hours. After cooling to room temperature the acetone was removed under reduced pressure, the residue was dissolved in ethyl acetate and extracted with water. The aqueous layers where extracted 3 times with ethyl acetate, the combined organic phases dried (Na_2SO_4) and evaporated to yield 56 g of the desiered compound as a colorless solid.

Yield: 56 g (96 %)

^{1}H-NMR (DMSO- d_6): 7.55-7.65 (m, 4H), 7.42 (t, J=8 Hz, 2H), 7.3 (t, J=8Hz, 1H), 6.95-7.05 (m, 2H), 4.07 (q, J=7Hz, 2 H), 1.35 (t, J=7Hz, 3H)

Intermediate 1B

2-bromo-1-(4'-ethoxy[1,1'-biphenyl]-4-yl)-1-ethanone

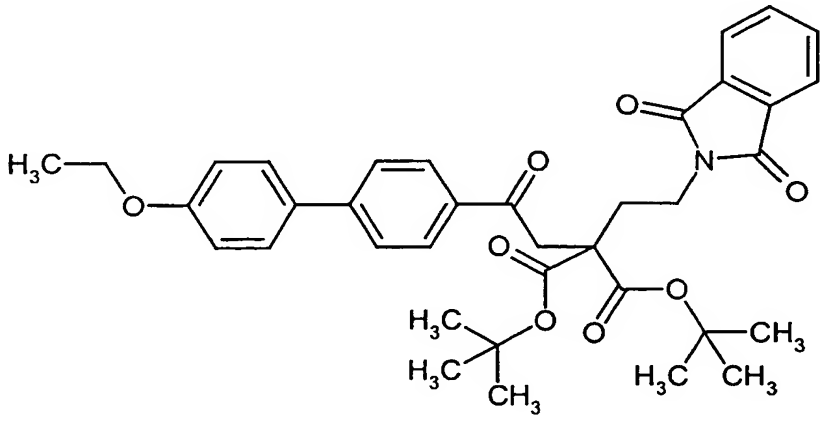


A solution of 56 g (282 mmol) of Intermediate 1A in 1.5 L CH_2Cl_2 was cooled to 0°C and placed under argon. Bromoacetyl bromide (85.5g, 36,8 mL, 423 mmol) was added, and then $AlCl_3$ (37.41 g, 280.53 mmol) was added in portions over 60 min. After the addition was complete, the mixture was stirred for 20 h, warming to RT. The mixture was then poured slowly into a stirred mixture of 2 kg ice/500ml conc. HCl. The organic layer was separated, , washed with 2N HCl and water, dried (Na_2SO_4) and evaporated. The crude product was purified by recrystallization (acetonitril) to give 49.3g (54%) white solid.

^{1}H-NMR (DMSO- d_6): 8.08 (d, J=8Hz, 2H), 7.81 (d, J=8Hz, 2H), 7.73 (d, J=8Hz, 2Hz), 7.06 (d, J=8Hz, 2H), 4.95 (s, 2H), 4.1 (q, J=7Hz, 2H), 1.36 (t, J=7Hz, 3H)

Intermediate 1C

Di(tert-butyl) 2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-2-[2-(4'-ethoxy-[1,1'-biphenyl]-4-yl)-2-oxoethyl]malonate



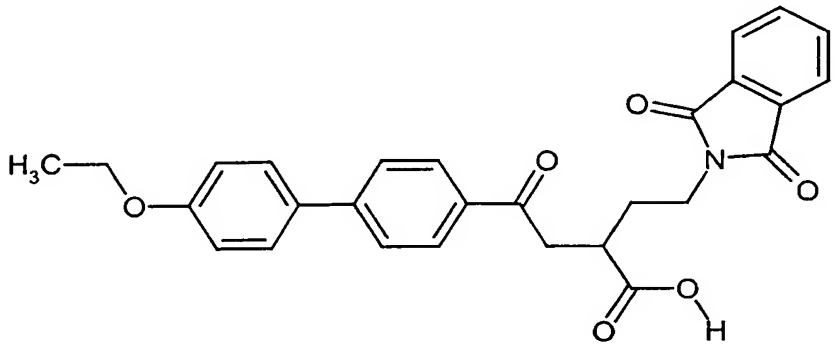
A solution of Intermediate 5F (3.2 g, 10 mmol) in 50 mL DMF was added dropwise to a suspension of NaH (500 mg, 12.5 mmol) in 20 mL DMF and stirred for 30 min at RT. Intermediate 1B (3.9 g, 10 mmol) in 30 ml DMF was added slowly and the resulting mixture was stirred for 4 h at RT. The reaction was quenched with saturated NH_4Cl solution, extracted twice with diethyl ether, washed with saturated $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The crude product was purified using flash chromatography (hexane / ethyl acetate : 1 / 1).

Yield: 4.96 g (71 %)

^{1}H-NMR (DMSO- d_6): 8.02 (d, J=8Hz, 2H), 7.68-7.81 (m, 8H), 7.05 (d, J=8Hz, 2H), 4.1 (q, J=7Hz, 2H), 3,72 (s, 2H), 3.55-3.65 (m, 2H), 2.26-2.87 (m, 2H), 1.33-1.4 (m, 18H)

Example 1

(rac)-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(4'-ethoxy[1,1'-biphenyl]-4-yl)-4-oxobutanoic acid



2.2 g (3.51 mmol) of Intermediate 1C was added in one portion to a cooled (0°C) 1:1 mixture of CH_2Cl_2 and trifluoroacetic acid. The reaction mixture was stirred overnight at RT, evaporated and dried under vacuum. The residue was disolved in 5 mL dioxane and heated for 5 h under reflux. The rection mixture was evaporated, the residue triturated with diethyl ether, stirred for 15 min and filtered. The remaining solid was dried under vacuum.

Yield: 1.21 g (73 %)

^{1}H-NMR (d_6-DMSO): 12.28 (s, 1H), 8.02 (d, J=8Hz, 2H), 7.67-7.9 (m, 8H), 7.05 (d, J=8Hz, 2H), 4.1 (q, J=7Hz, 2H), 3.72 (t, J=7.5Hz, 2H), 3.49 (dd, J=17.5Hz, J=8Hz, 2H), 3.28 (dd, J=17.5Hz, J=5Hz, 2H), 2.78-2.95 (m,1H), 1.76-2.13 (m, 2H), 1.38 (t, J=7Hz, 3H)

The racemate of Example 1 was separated into its pure enantiomers via chiral HPLC using a commercially available 5 µm Kromasil KR 100-5-CHI-DMB phase. A solvent mixture consisting of 50% iso-hexane and 50% of a tert-butylmethyl ether / dichloromethane / glacial acetic acid mixture (480:40:1) was employed at a constant flow rate of 25 ml/min.

Example 2

(+)-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(4'-ethoxy[1,1'-biphenyl]-4-yl)-4-oxobutanoic acid

Faster eluting enantiomer:
Yield: 374 mg (42 %)
$[\alpha]^{23}_D$ + 6.33° (c=0.47 in THF)

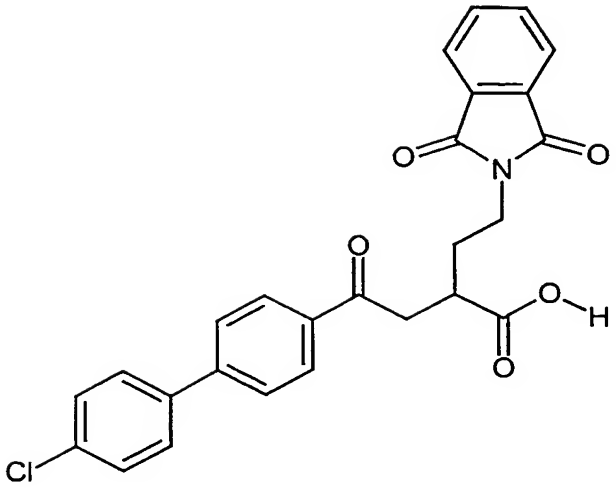
Example 3

(-)-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(4'-ethoxy[1,1'-biphenyl]-4-yl)-4-oxobutanoic acid

Slower eluting enantiomer:
Yield: 321 mg (36%)
$[\alpha]^{23}_D$ - 5.6° (c=0.5 in THF)

Example 4

(+)-4-(4'-chloro[1,1'-biphenyl]-4-yl)-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-oxobutanoic acid



The compound of Example 4 was prepared according to the procedure given for Example 268 in WO 96/15096.

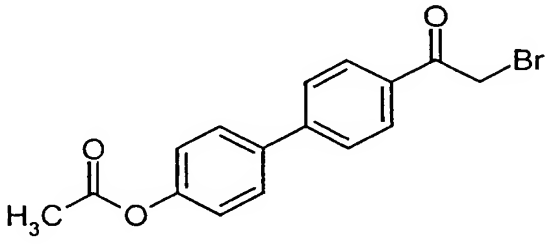
$[\alpha]^{23}_D$ + 5.55° (c=0.525 in THF)

Example 5

(rac)-4-[4'-(Acetyloxy)[1,1'-biphenyl]-4-yl]-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-ethyl]-4-oxobutanoic acid

Intermediate 5A

4'-(2-Bromoacetyl)[1,1'-biphenyl]-4-yl acetate



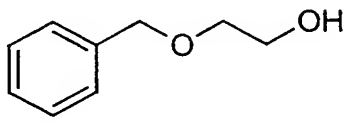
A solution of 50 g (236 mmol) of [1,1'-biphenyl]-4-yl acetate in 500 ml dichloromethane was placed under argon and cooled to 0°C. Bromoacetyl bromide (31.6 ml, 363 mmol) was added, followed by aluminium chloride (94.3 g, 707 mmol) which was added in portions under vigorous stirring over 30 min. The resulting mixture was stirred at 0°C for a further 30 min and at room temperature overnight. The mixture was then slowly poured into 500 ml of cold 10% HCl and extracted three times with dichloromethane. The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was triturated with 1:1 diisopropyl ether / isopropanol, filtered, and the remaining solid dried under vacuum.

Yield: 73.3 g (93.4%)

^{1}H-NMR ($CDCl_3$): δ = 2.34 (s, 3 H), 4.48 (s, 2 H), 7.21 (m, 2 H), 7.66 (m, 4 H), 8.08 (m, 2 H).

Intermediate 5B

2-(Benzyloxy)-1-ethanol



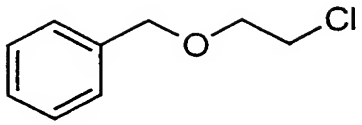
Ethylene glycol (742.5 g, 11.96 mol) was added to a solution of sodium hydroxide pellets (475.2 g, 11.88 mol) in 450 ml of water kept at 80°C. Benzyl chloride (302.8 g, 2.39 mol) was then added at 65°C, and the resulting suspension was vigorously stirred at 120°C overnight. After cooling to room temperature, the mixture was poured into ice-water and extracted five times with diethyl ether. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated. The remaining residue was then distilled under vacuum and the relevant fractions (bp. 95-125°C at 0.1-1 mbar) collected.

Yield: 175 g (48.1%) of a colourless liquid.

^{1}H-NMR ($CDCl_3$): δ = 2.09 (tr, 1 H), 3.60 (m, 2 H), 3.77 (m, 2 H), 4.56 (s, 2 H), 7.35 (m, 5 H).

Intermediate 5C

Benzyl 2-chloroethyl ether



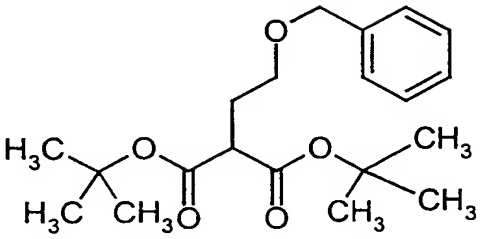
Thionyl chloride (41.2 ml, 567.6 mmol) was slowly added to a mixture of Intermediate 5B (90 g, 80% purity, 473.1 mmol) and N,N-dimethyl aniline (76.5 ml, 597.5 mmol) while keeping the reaction temperature at 50°C by ice-water cooling. After stirring at 50°C for 1 h, further portions of N,N-dimethyl aniline (15.3 ml, 119.5 mmol) and thionyl chloride (8.2 ml, 113.5 mmol) were added, and the mixture was stirred at 50°C for another 2 h and at room temperature overnight. The solution was then poured into a mixture of ice-water (200 ml) and conc. HCl (100 ml) and extracted three times with dichloromethane. The combined organic layers were washed twice with 10% HCl and twice with water, dried over Na_2SO_4, filtered and evaporated. The remaining residue was then distilled under vacuum (water pump) and the relevant fractions collected.

Yield: 69.1 g (85.6%) of a colourless liquid.

^{1}H-NMR ($CDCl_3$): δ = 3.69 (m, 4 H), 4.59 (s, 2 H), 7.35 (m, 5 H).

Intermediate 5D

Di(tert-butyl) 2-[2-(benzyloxy)ethyl] malonate



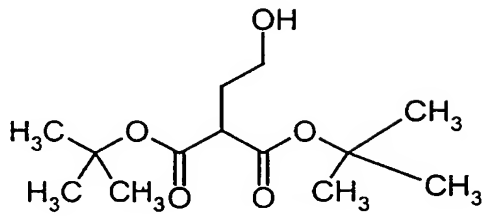
Di(tert-butyl) malonate (151.4 g, 686 mmol) was added dropwise at 50°C to a suspension of potassium tert-butylate (77 g, 686 mmol) in 500 ml of tert-butanol. Sodium iodide (10.33 g) was then added, followed by dropwise addition of Intermediate 5C (117.1 g, 686 mmol) at 40-50°C. The resulting thick suspension was stirred at 70°C for two days. During this time, two further portions of potassium tert-butylate (15.4 g each, 70 mmol) were added. The mixture was then poured into ice-water and extracted three times with diethyl ether. The organic layers were dried over Na_2SO_4, filtered and evaporated. The crude product was finally purified by column chromatography using a cyclohexane / ethyl acetate gradient (70:1 -> 15:1).

Yield: 134 g (55.8%) of a colourless oil.

^{1}H-NMR (DMSO-d_6): δ = 1.38 (s, 18 H), 1.96 (q, 2 H), 3.31 (tr, 1 H), 3.41 (tr, 2 H), 4.43 (s, 2 H), 7.31 (m, 5 H).

Intermediate 5E

Di(tert-butyl) 2-(2-hydroxyethyl) malonate



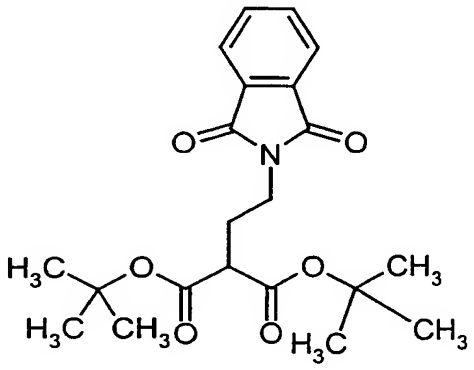
A solution of Intermediate 5D (46.58 g, 132.9 mmol) in 300 ml ethanol was hydrogenated at atmospheric pressure in the presence of 10% palladium on charcoal (2.0 g). After stirring for 3 h at room temperature, another 1.0 g portion of palladium catalyst was added, and stirring was continued at room temperature overnight. The mixture was then filtered through celite, evaporated, and the crude product purified by column chromatography using a dichloromethane / methanol gradient (70:1 -> 30:1).

Yield: 23.3 g (67.2%) of a pale yellow oil.

^{1}H-NMR ($CDCl_3$): δ = 1.47 (s, 18 H), 1.96 (tr, 1 H), 2.08 (q, 2 H), 3.36 (tr, 1 H), 3.72 (q, 1 H).

Intermediate 5F

Di(tert-butyl) 2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] malonate



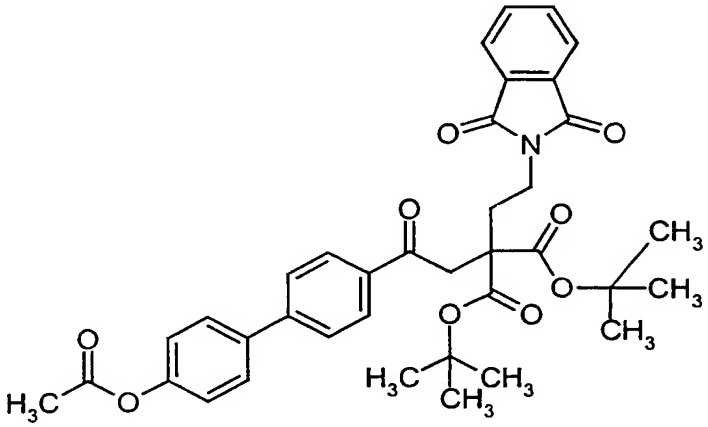
To a stirred solution of Intermediate 5E (30.0 g, 115.2 mmol) in 255 ml of dry THF were added successively phthalimide (21.4 g, 144.1 mmol), triphenyl phosphine (35.1 g, 132.5 mmol) and, at 0°C, diethyl azodicarboxylate (22.1 g, 126.8 mmol). The resulting solution was stirred overnight while warming up to room temperature, then diluted with ethyl acetate and washed twice with water and with brine. The organic phase was dried over Na_2SO_4, filtered and evaporated. The crude product was finally purified by column chromatography using a cyclohexane / dichloromethane / ethyl acetate gradient (7:1:1 - > 5:1:1).

Yield: 10.02 g (22.3%) of a white solid.

^{1}H-NMR (DMSO-d_6): δ = 1.37 (s, 18 H), 2.03 (q, 2 H), 3.30 (tr, 1 H), 3.63 (tr, 2 H), 7.85 (m, 4 H).

Intermediate 5G

Di(tert-butyl) 2-{2-[4'-(acetyloxy)[1,1'-biphenyl]-4-yl]-2-oxoethyl}-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] malonate



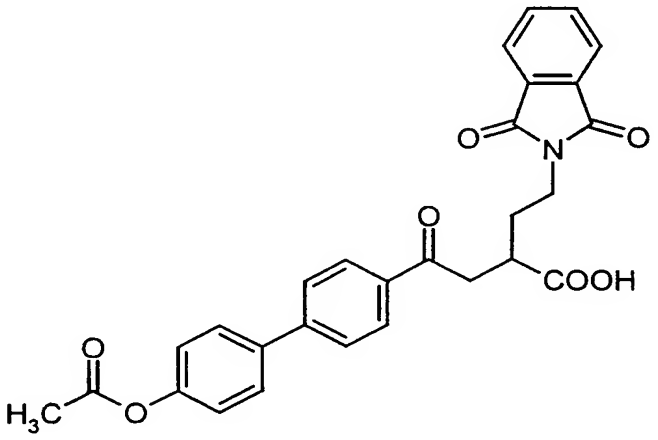
Under argon, a solution of Intermediate 5F (6.5 g, 16.69 mmol) in 60 ml of dry THF was added dropwise at 0°C to a suspension of sodium hydride (0.51 g, 80% suspension in mineral oil, 16.86 mmol) in 30 ml of dry THF. After stirring at 30-40°C for 30 min, the mixture was re-cooled to 0°C, and a solution of Intermediate 5A (5.6 g, 16.86 mmol) in 60 ml of dry THF was added dropwise. The mixture was then stirred overnight while warming up to room temperature. Further portions of sodium hydride (0.1 g, 3.4 mmol) and Intermediate 5A (1.12 g, 3.4 mmol) were added at 0°C, and stirring was continued at room temperature for another 3 h. The reaction mixture was quenched by addition of saturated ammonium chloride solution (100 ml) and brine (200 ml), and extracted twice with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered and evaporated. The crude product was finally purified by column chromatography using a dichloro-methane / ethyl acetate gradient (50:1 -> 30:1).

Yield: 4.41 g (41.2%) of an off-white solid.

^{1}H-NMR (DMSO-d_6): δ = 1.38 (s, 18 H), 2.32 (m, 5 H), 3.61 (tr, 2 H), 3.73 (s, 2 H), 7.28 (d, 2 H), 7.81 (m, 8 H), 8.04 (d, 2 H).

Example 5

(rac)-4-[4'-(Acetyloxy)[1,1'-biphenyl]-4-yl]-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-oxobutanoic acid



Intermediate 5G (400 mg, 0.62 mmol) was dissolved at 0°C in a mixture of dichloromethane (5 ml) and trifluoroacetic acid (5 ml). After stirring at room temperature for 1.5 h, 10 ml of toluene were added, and the reaction mixture was evaporated. The residue was dried under vacuum, then re-dissolved in 20 ml of dioxane, and the solution heated under reflux for 6 h. The mixture was evaporated to dryness, the residue triturated with diethyl ether, filtered, and the remaining solid dried under vacuum to give the final product.

Yield: 261 mg (86.2%) of an off-white solid.

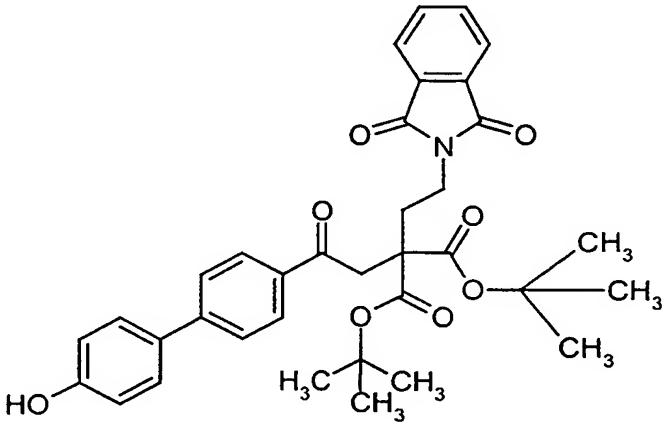
^{1}H-NMR (DMSO-d_6): δ = 1.95 (m, 2 H), 2.31 (s, 3 H), 2.89 (m, 1 H), 3.38 (m, 2 H), 3.72 (tr, 2 H), 7.28 (d, 2 H), 7.84 (m, 8 H), 8.07 (d, 2 H), 12.33 (br s, 1 H).

Example 6

(rac)-2-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(4'-hydroxy[1,1'-biphenyl]-4-yl)-4-oxobutanoic acid

Intermediate 6A

Di(tert-butyl) 2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-2-[2-(4'-hydrox-y-[1,1'-biphenyl]-4-yl)-2-oxoethyl] malonate



Finely powdered, anhydrous potassium carbonate (2.15 g, 15.58 mmol) was added to a solution of Intermediate 5G (2.0 g, 3.12 mmol) in 90 ml of a THF / methanol / ethanol mixture (30:50:10). The resulting suspension was vigorously stirred at room temperature for 45 min, then diluted with ethyl acetate and filtered. The filtrate was concentrated under vacuum to half of its original volume, diluted again with ethyl acetate, and then poured into an ice-cold pH 4 buffer solution. The aqueous phase was extracted twice with ethyl acetate, and the combined organic layers were dried over Na_2SO_4, filtered and evaporated. After drying under vacuum, 700 mg (approx. 1.1 mmol) of the residue (which contained some ring-opened material) were dissolved in 20 ml of dichloromethane, and 1-hydroxy-1H-benzotriazol hydrate (189 mg, 1.23 mmol) and N'-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (229 mg, 1.18 mmol) were added at 0°C. The reaction mixture was stirred at room temperature for 3 days, then diluted with dichloromethane, and washed twice

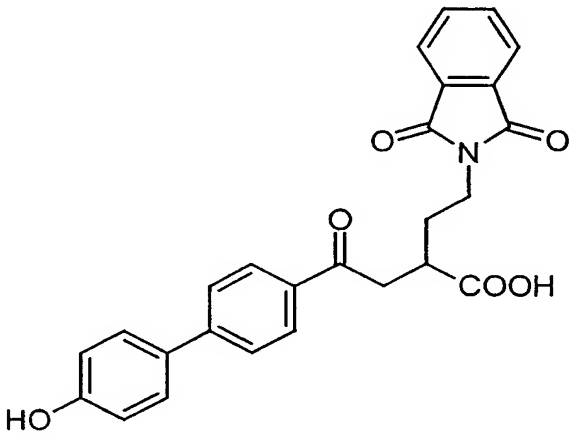
with pH 4 buffer solution and with saturated sodium hydrogencarbonate solution. The organic phase was dried over Na_2SO_4, filtered and evaporated. The crude product was finally purified by column chromatography using a dichloromethane / methanol gradient (100:1 -> 80:1).

Yield: 494 mg (71%) of a white solid.

^{1}H-NMR (DMSO-d_6): δ = 1.38 (s, 18 H), 2.31 (m, 2 H), 3.60 (m, 2 H), 3.70 (s, 2 H), 6.90 (d, 2 H), 7.61 (d, 2 H), 7.78 (m, 6 H), 8.00 (d, 2 H), 9.76 (s, 1 H).

Example 6

(rac)-2-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-(4'-hydroxy[1,1'-biphen-yl]-4-yl)-4-oxobutanoic acid



Intermediate 6A (490 mg, 0.82 mmol) was dissolved at 0°C in a mixture of dichloromethane (7.5 ml) and trifluoroacetic acid (7.5 ml). After stirring at room temperature for 30 min, 7 ml of toluene were added, and the reaction mixture was evaporated. The residue was dried under vacuum, then re-dissolved in 15 ml of dioxane, and the solution heated under reflux for 4.5 h. After cooling to room temperature, diethyl ether (15 ml) was added to the reaction mixture, and the precipitated product collected by filtration. The filtrate was evaporated to dryness, the residue triturated

with diethyl ether, containing a few drops of methanol, and filtered again to give a second crop of the final product.

Yield: 299 mg (82.6%) of a white solid.

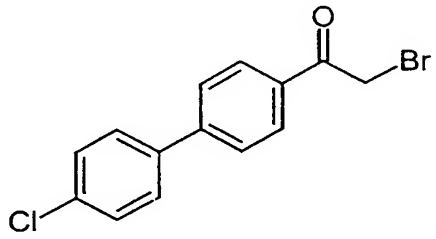
^{1}H-NMR (DMSO-d_6): δ = 1.94 (m, 2 H), 2.87 (m, 1 H), 3.38 (m, 2 H), 3.70 (tr, 2 H), 6.89 (d, 2 H), 7.61 (d, 2 H), 7.75 (d, 2 H), 7.85 (m, 4 H), 8.01 (d, 2 H), 9.76 (br s, 1 H), 12.30 (br s, 1 H).

Example 7

(rac)-4-(4'-Chloro[1,1'-biphenyl]-4-yl)-2-[2-(4,6-dimethoxy-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-oxobutanoic acid

Intermediate 7A

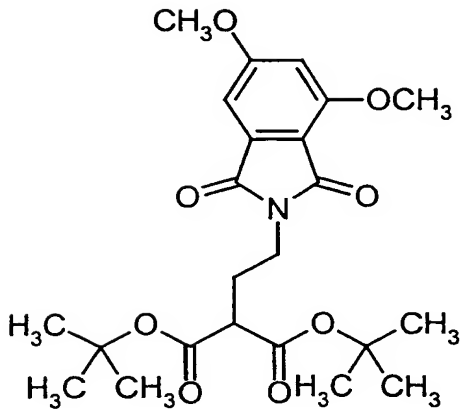
2-Bromo-1-(4'-chloro[1,1'-biphenyl]-4-yl)-1-ethanone



This intermediate was prepared as described in the indicated reference WO 96/15096.

Intermediate 7B

Di(tert-butyl) 2-[2-(4,6-dimethoxy-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] malonate



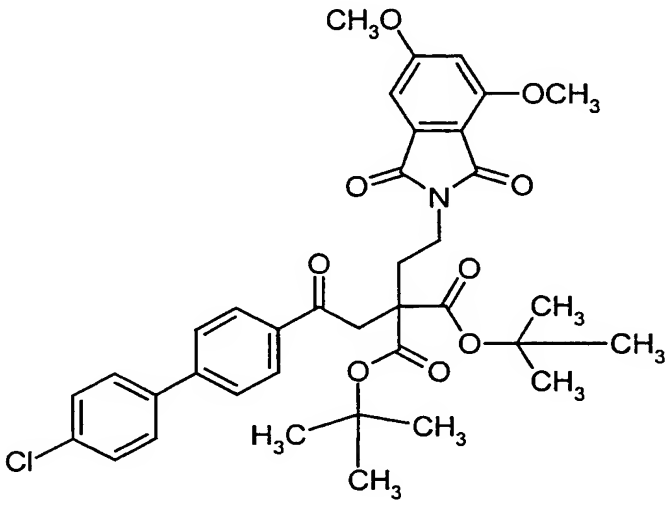
To a stirred solution of Intermediate 5E (8.4 g, 32.2 mmol) in 100 ml of dry THF were added successively 3,5-dimethoxyphthalimide (10.0 g, 48.3 mmol), triphenyl phosphine (11.1 g, 41.8 mmol) and, at 0°C, diethyl azodicarboxylate (6.7 g, 38.6 mmol). The resulting solution was stirred overnight while warming up to room temperature. After filtration, the filtrate was diluted with ethyl acetate and washed twice with water and with brine. The organic phase was dried over Na_2SO_4, filtered and evaporated. The crude product was finally purified by column chromatography using a dichloromethane / ethyl acetate gradient (70:1 -> 30:1).

Yield: 2.69 g (18.6%) of a white solid.

^{1}H-NMR (DMSO-d_6): δ = 1.38 (s, 18 H), 1.97 (q, 2 H), 3.23 (tr, 1 H), 3.54 (tr, 2 H), 3.92 (s, 6 H), 6.89 (d, 1 H), 6.97 (d, 1 H).

Intermediate 7C

Di(tert-butyl) 2-[2-(4'-chloro[1,1'-biphenyl]-4-yl)-2-oxoethyl]-2-[2-(4,6-dimethoxy-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] malonate



Under argon, a solution of Intermediate 7B (2.69 g, 5.98 mmol) in 30 ml of dry THF was added dropwise at 0°C to a suspension of sodium hydride (0.18 g, 80% suspension in mineral oil, 6.04 mmol) in 20 ml of dry THF. After stirring at 30-40°C for 30 min, the mixture was re-cooled to 0°C, and a solution of Intermediate 3A (1.87 g, 6.04 mmol) in 20 ml of dry THF was added dropwise. The mixture was then stirred overnight while warming up to room temperature. Further portions of sodium hydride (36 mg, 1.2 mmol) and Intermediate 7A (374 mg, 1.2 mmol) were added at 0°C, and stirring was continued at room temperature for three days. The reaction mixture was quenched by addition of saturated ammonium chloride solution (40 ml) and brine (80 ml), and extracted twice with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered and evaporated. The crude product was finally purified by column chromatography using a dichloro-methane to dichloromethane / ethyl acetate (20:1) gradient.

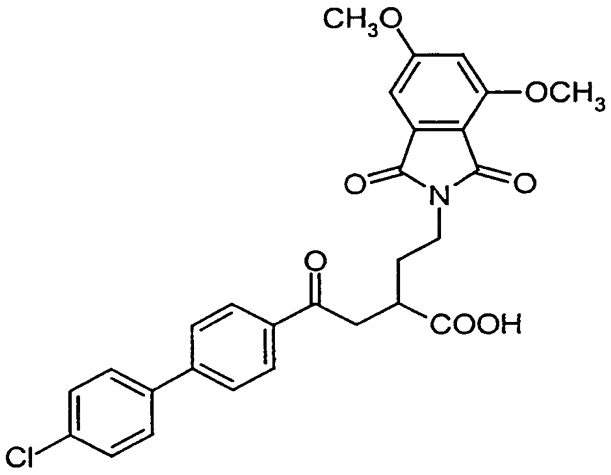
Yield: 1.51 g (37.2%) of a white solid.

R_F = 0.51 (dichloromethane / ethyl acetate 20:1)
= 0.59 (cyclohexane / ethyl acetate 1:1)

ESI-MS: m/z = 678 $[M+H]^+$, 622 ($[M+H]^+$-C_4H_8), 566 ($[M+H]^+$-2xC_4H_8), 548 ($[M+H]^+$-2xC_4H_8/-H_2O).

Example 7

(rac)-4-(4'-Chloro[1,1'-biphenyl]-4-yl)-2-[2-(4,6-dimethoxy-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-oxobutanoic acid



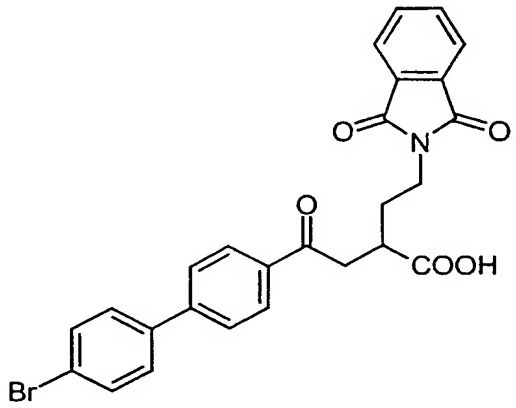
Intermediate 7C (1.5 g, 2.2 mmol) was dissolved at 0°C in a mixture of dichloromethane (10 ml) and trifluoroacetic acid (10 ml). After stirring at room temperature for 45 min, 10 ml of toluene were added, and the reaction mixture was evaporated. The residue was dried under vacuum, then re-dissolved in 20 ml of dioxane, and the solution heated under reflux for 6 h. The mixture was evaporated to dryness, the residue triturated with diethyl ether, filtered, and the remaining solid dried under vacuum to give the final product.

Yield: 1.07 g (92.1%) of an off-white solid.

^{1}H-NMR (DMSO-d_6): δ = 1.89 (m, 2 H), 2.84 (m, 1 H), 3.39 (m, 2 H), 3.63 (tr, 2 H), 3.92 (s, 6 H), 6.88 (d, 1 H), 6.97 (d, 1 H), 7.58 (d, 2 H), 7.82 (m, 4 H), 8.07 (d, 2 H), 12.30 (br s, 1 H).

Examples 8 and 9

(+)- and (-)-4-(4'-Bromo[1,1'-biphenyl]-4-yl)-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl]-4-oxobutanoic acid



Racemic 4-(4'-Bromo[1,1'-biphenyl]-4-yl)-2-[2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)- ethyl]-4-oxobutanoic acid was prepared essentially as described in the indicated reference WO 96/15096.

^{1}H-NMR (DMSO-d_6): δ = 1.95 (m, 2 H), 2.88 (m, 1 H), 3.38 (m, 2 H), 3.72 (tr, 2 H), 7.72 (m, 4 H), 7.85 (m, 6 H), 8.08 (d, 2 H), 12.33 (br s, 1 H).

1.0 g (1.97 mmol) of this material was separated into pure enantiomers by chiral HPLC using a commercially available 5 µm Kromasil KR 100-5-CHI-DMB phase. A solvent mixture consisting of 40% iso-hexane and 60% of a tert-butylmethyl ether / dichloromethane / glacial acetic acid mixture (480:40:1) was employed at a constant flow rate of 25 ml/min.

Example 8

First eluting enantiomer A:

Yield: 309 mg (31%)

$[\alpha]_D^{20}$ = +3.87° (c = 0.458 g / 100 ml, THF)

Example 9

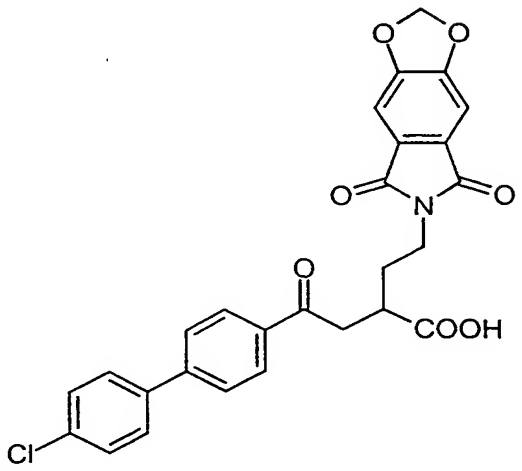
Second eluting enantiomer B:

Yield: 240 mg (24%)

$[\alpha]_D^{20}$ = -5.98° (c = 0.482 g / 100 ml, THF)

Examples 10 and 11

(+)- and (-)-4-(4'-Chloro[1,1'-biphenyl]-4-yl)-2-[2-(5,7-dioxo-5,7-dihydro-6H-[1,3]-dioxolo[4,5-f]isoindol-6-yl)ethyl]-4-oxobutanoic acid



Racemic 4-(4'-Chloro[1,1'-biphenyl]-4-yl)-2-[2-(5,7-dioxo-5,7-dihydro-6H-[1,3]-dioxolo-[4,5-f]isoindol-6-yl)ethyl]-4-oxobutanoic acid was prepared essentially as described in the indicated reference WO 96/15096.

^{1}H-NMR (DMSO-d_6): δ = 1.91 (m, 2 H), 2.85 (m, 1 H), 3.38 (m, 2 H), 3.66 (tr, 2 H), 6.26 (s, 2 H), 7.39 (s, 2 H), 7.58 (d, 2 H), 7.82 (m, 4 H), 8.06 (d, 2 H), 12.31 (br s, 1 H).

0.70 g (1.38 mmol) of this material was separated into pure enantiomers by chiral HPLC using a commercially available 5 µm Kromasil KR 100-5-CHI-MDB phase. A solvent mixture consisting of 40% iso-hexane and 60% of a tert-butylmethyl ether / dichloromethane / glacial acetic acid mixture (480:40:1) was employed at a constant flow rate of 25 ml/min.

Example 10

First eluting enantiomer A:

Yield: 261 mg (37.3%)

$[\alpha]_D^{20}$ = +13.99° (c = 0.955 g / 100 ml, THF)

Example 11

Second eluting enantiomer B:

Yield: 228 mg (32.6%)

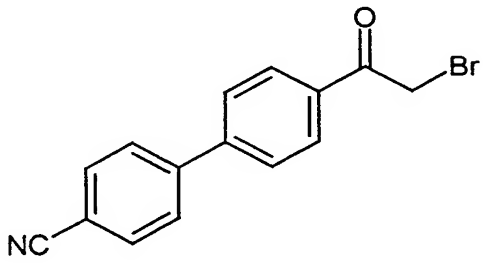
$[\alpha]_D^{20}$ = -15.15° (c = 0.991 g / 100 ml, THF)

Example 12

(rac)-4-(4'-Cyano[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}butanoic acid

Intermediate 12A

4'-(2-bromoacetyl)[1,1'-biphenyl]-4-carbonitrile

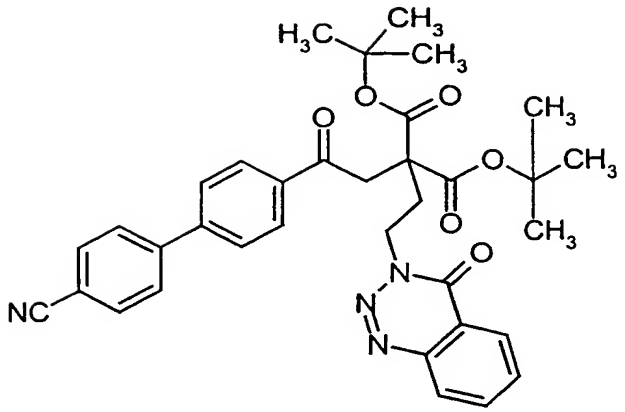


4.68 g aluminium chloride (35.15 mmol) are dissolved in 45 ml dichloromethane and treated dropwise with 3.38 g (16.74 mmol) bromoacetyl bromide at 0°C. After 30 min 3 g (16.74 mmol) 4-cyanobiphenyl, dissolved in 15 ml dichloromethane, are added dropwise. The reaction mixture is stirred overnight at ambient temperature, added to ice-water and extracted 2 times with dichloromethane. The organic phase is washed with water and brine, dried and evaporated. The residue is triturated with petrol ether, filtered and dried. Yield: 4.24 g (83%).

200 MHz ^{1}H-NMR ($CDCl_3$): 4.49, s, 2H; 7.73, m, 6H; 8.11, d, 2H.

Intermediate 12B

Di(tert-butyl)-2-[2-(4'-cyano[1,1'-biphenyl]-4-yl)-2-oxoethyl]-2-{2-[4-oxo-1,2,3-benzo-triazin-3(4H)-yl]ethyl}malonate

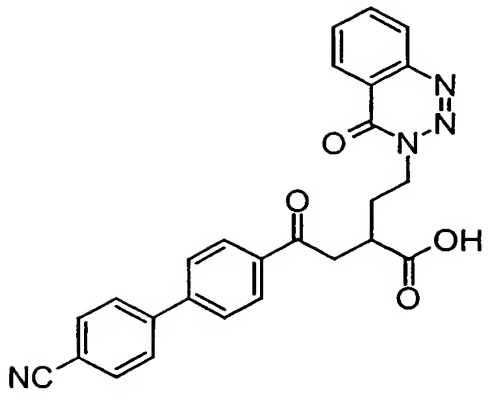


A solution of 2.59 g (6.67 mmol) of Intermediate 13C in 20 ml DMF is added dropwise to a suspension of 0.333 g NaH (60% in mineral oil) in 20 ml DMF. The mixture is stirred for 30 min and a solution of 2 g (6.67 mmol) of Intermediate 12A in 20 ml DMF is added. The mixture is stirred for 2.5h at RT, poured onto 150 ml NH_4Cl solution and extracted with ethyl acetate. The organic phase is washed with water and brine, dried over $MgSO_4$ and evaporated. The residue is purified by chromatography to give 0.62 g (15%).

200 MHz ^{1}H-NMR ($CDCl_3$): 2.71, s, 2H; 3.81, s, 2H; 4.53, m, 2H; 7.75, m, 7H; 7.91, m, 1H; 8.10, m, 3H; 8.30, m, 1H.

Example 12

(rac)-4-(4'-Cyano[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}butanoic acid



0.61 g (1 mmol) of Intermediate 12B is dissolved in 10 ml dichloro-methane / trifluoroacetic acid (1:1) at 0°C and the mixture is stirred for 2h at RT. After adding toluene the reaction mixture is evaporated to dryness and the residue taken into 10 ml dioxane. The solution is stirred under reflux for 6h and at RT overnight. The solvent is removed in vacuo and the residue purified by HPLC to yield 96 mg (21%).

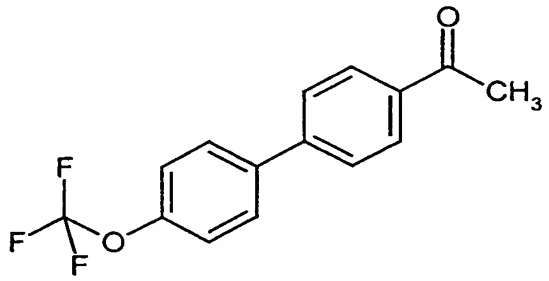
200 MHz ^{1}H-NMR ($CDCl_3$): 2.28, m, 2H; 2.49, m, 2H; 3.26, m, 1H; 4.58, m, 2H, 7.72, m, 10H; 8.18, m, 1H; 8.38, m, 1H.

Example 13

(rac)-4-Oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}-4-[4'-(trifluoromethoxy)[1,1'-biphenyl]-4-yl]butanoic acid

Intermediate 13A

1-[4'-(Trifluoromethoxy)[1,1'-biphenyl]-4-yl]-1-ethanone

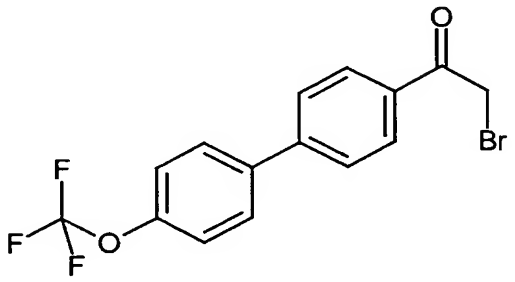


To a mixture of 30 g (126 mmol) 4-(trifluoromethoxy)-1,1'-biphenyl and 21 g aluminium trichloride (157 mmol) in 120 ml nitrobenzene are added at a temperature below 20°C 9.87 g (126 mmol) acetyl chloride. The reaction mixture is stirred for 2h at 0°C, added to 240 ml ice-water and 42 ml conc. HCl and extracted with ethyl acetate. The organic phase is washed with water and brine and the solvents removed in vacuo. The residue is triturated with petrol ether, filtrated and dried. From the filtrate another batch can be obtained after crystallizing at 4°C to give overall 23.5 g (66%).

200 MHz ^{1}H-NMR ($CDCl_3$): 2.65, s, 3H; 7.33, d, 2H; 7.58, m, 4H; 8.06, d, 2H.

Intermediate 13B

2-Bromo-1-[4'-(trifluoromethoxy)[1,1'-biphenyl]-4-yl]-1-ethanone

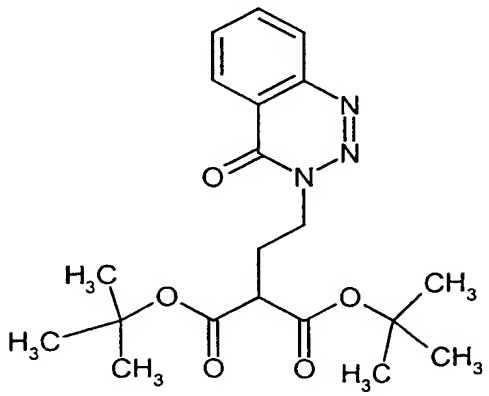


12.27 g (43.8 mmol) of Intermediate 13A are dissolved in a mixture of 150 ml methanol, 150 ml ethanol and 50 ml ether with gentle heating. 5.914 g (56.9 mmol) boronic acid trimethylester are added at RT and 7.35 g (45.9 mmol) bromine are added dropwise. The reaction mixture is stirred until disappearance of the red-brown colour. Solvents are removed in vacuo and the residue is purified by chromatography (cyclohexane/ethyl acetate) to give 6.2 g (39%).

200 MHz ^{1}H-NMR ($CDCl_3$): 4.49, s, 2H; 7.33, d, 2H; 7.68, dd, 4H; 8.10, d, 2H.

Intermediate 13C

Di(tert-butyl) 2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}malonate



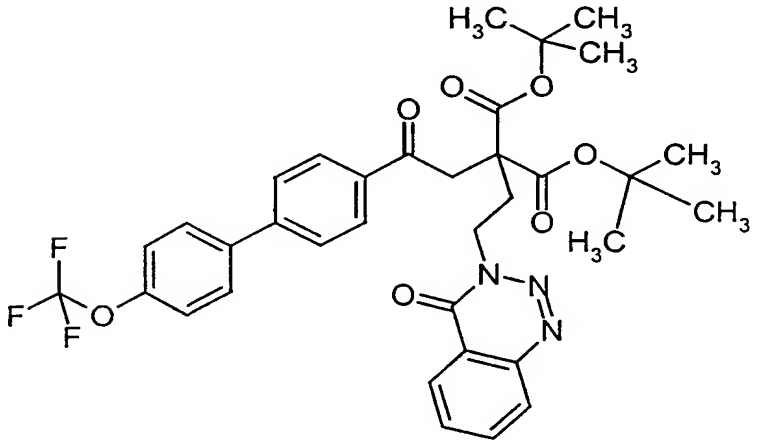
46.2 g (177 mmol) of Intermediate 5E are dissolved in 600 ml THF. 69.8 g (266 mmol) triphenylphosphin und 39.2 g (266 mmol) 1,2,3-benzotriazin-4(3H)-one are added. 46.4 g (266 mmol) DEAD are added dropwise. The reaction mixture was stirred overnight at roomtemperature. The solvent is removed in vacuo and the product obtained by chromatography (cyclohexan/ethylacetate 6:1).

Yield: 51.8g (63%).

200 MHz ^{1}H-NMR ($CDCl_3$): 1.43, s, 18H; 2.43, quar., 2H; 3.30, t, 1H; 4.57, t, 2H; 7.80, m, 1H; 7.94, m, 1H; 8.16, dd, 1H; 8.36, dd, 1H.

Intermediate 13D

Di(tert-butyl)-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}-2-{2-oxo-2-[4'-(trifluoromethoxy)[1,1'-biphenyl]-4-yl]ethyl}malonate



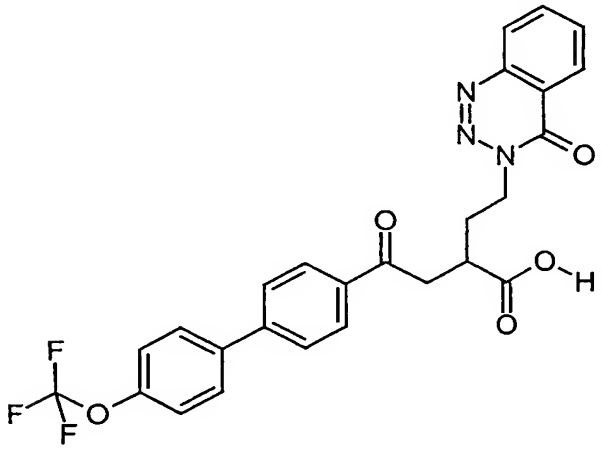
To a suspension of 0.52 g (12.9 mmol) sodium hydride (60% suspension in mineral oil) in 20 ml DMF is added a solution of 4.04 g (10.37 mmol) of Intermediate 13C in 30 ml DMF dropwise. After stirring for 30 min at RT a solution of 3.73 g (10.37 mmol) of Intermediate 13B in 30 ml DMF is added dropwise and the reaction mixture is stirred for 2h at RT. The reaction mixture is poured onto NH_4Cl solution, extracted with ethyl acetate and the organic phase is washed with water and brine.

After drying and evaporation of the solvents the product is purified by chromatography to give 2.09 g (30%).

200 MHz ^{1}H-NMR ($CDCl_3$): 2.70, m, 2H; 3.82, s, 2H; 4.54, m, 2H; 7.32, d, 2H; 7.62, m, 5H; 8.07,m, 4H; 8.30, m, 1H.

Example 13

(rac)-4-Oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}-4-[4'-(trifluoro-methoxy)[1,1'-biphenyl]-4-yl]butanoic acid

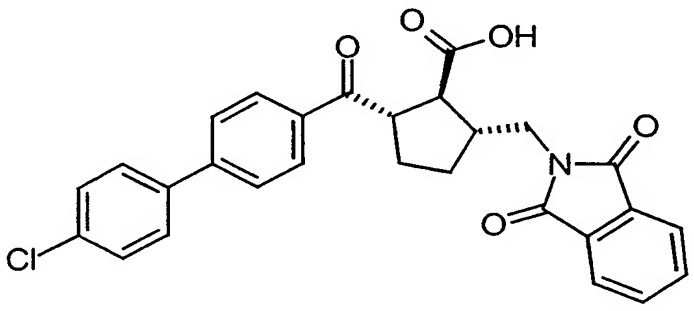


A solution of 2.09 g (3.13 mmol) of Intermediate 13C in 15 ml dichloromethane and 15 ml trifluoroacetic acid is stirred at RT for 2h. After adding toluene the solvents are removed in vacuo. The residue is dissolved in 30 ml dioxane, the solution is refluxed for 6h and stirred at RT overnight. Solvents are removed in vacuo, the residue is triturated with ether, the precipitate is collected by filtration and dried to give 0.51 g (32%).

200 MHz ^{1}H-NMR (DMSO-d_6): 2.18, m, 2H; 2.98, m, 1H; 3.51, m, 2H; 4.52, m, 2H; 7.52, d, 2H; 7.89, m, 5H; 8.10, m, 3H; 8.24, m, 2H; 12.39, s, 1H.

Example 14 and 15

(+)- and (-)-(1S, 2S, 5R)-2-[(4'-Chloro[1,1'-biphenyl]-4-yl)carbonyl]-5-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]cyclopentanecarboxylic acid



The racemic compound was prepared essentially following the procedure for Example 360 of WO 96/15096.

4.20 g (8.61 mmol) of this material was separated into pure enantiomers by chiral HPLC using a commercially available 5 µm Kromasil KR 100-5-CHI-MDB phase. A solvent mixture consisting of 30% iso-hexane and 70% of a tert-butylmethyl ether /dichloromethane/glacial acetic acid mixture (480:40:1) was employed at a constant flow rate of 25 ml/min.

Example 14

First eluting enantiomer A:

Yield: 1.72 g (41.0%)

$[\alpha]_D^{21}$ = +44.26° (c=0.464 g/100 ml, THF)

Example 15

Second eluting enantiomer B:

Yield: 1.59 g (37.9%)

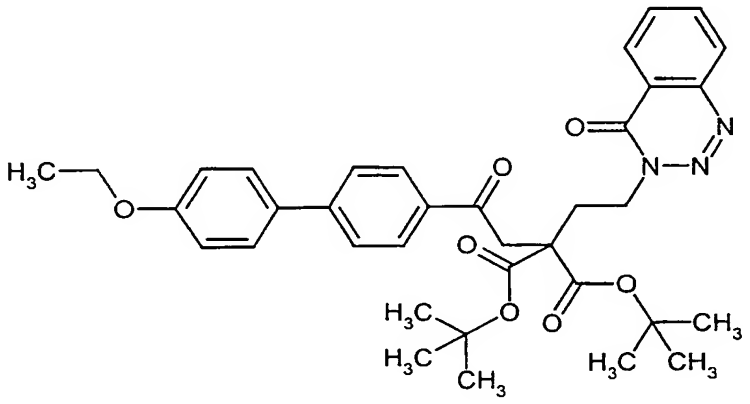
$[\alpha]_D^{21} = 43.70°$ (c = 0.575 g/100 ml, THF)

Example 16

(rac)-4-(4'-Ethoxy-biphenyl-4-yl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-4-oxo-butyric acid

Intermediate 16 A

tert.-Butyl-4-(4'-ethoxy-biphenyl-4-yl)-4-oxo-2-(tert.-butyloxycarbonyl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-butanoate



A solution of Intermediate 13C (1.29 g, 3.32 mmol) in 15 mL DMF was added dropwise to a suspension of NaH (170 mg, 4.15 mmol) in 5 mL DMF and stirred for 30 min at rt. Intermediate 1B (1.06 g, 3.32 mmol) in 15 ml DMF was added slowly and the resulting mixture was stirred for 2.5 h at rt. The reaction was quenched with saturated NH_4Cl solution, extracted twice with diethyl ether, washed with saturated

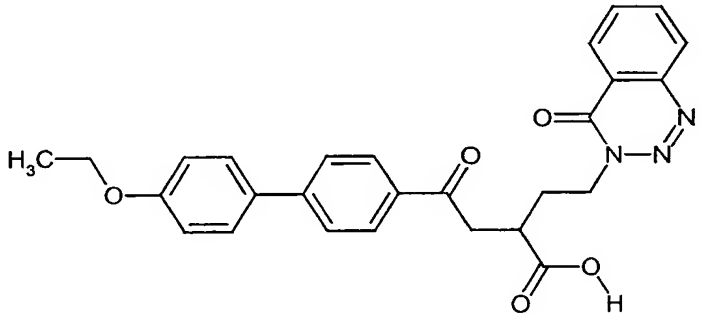
$NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The crude product was purified using flash chromatography (Hexane / Ethyl acetate : 9/1-> 5/1).

Yield: 1.34 g (59 %)

^{1}H-NMR (D_6-DMSO): 7.65-8.25 (m, 10H), 7.05 (d, J=8Hz, 2H), 4.39-4.51 (m, 2H), 4.1 (q, J=7Hz, 2H), 3,75 (s, 2H), 2.45-2.55 (m, 2H), 1.33-1.4 (m, 21H)

Example 16

(rac)-4-(4'-Ethoxy-biphenyl-4-yl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-4-oxo-butyric acid



1.31 g (2.09 mmol) of Intermediate 16A were added in one portion to a cooled (0°C) 1:1 mixture of CH_2Cl_2 and trifluoroacetic acid (20 mL). The reaction mixture was stirred for 2 h at rt, evaporated and dried under vacuum. The residue was disolved in 20 mL dioxane and heated for 5 h under reflux. The rection mixture was evaporated, the residue triturated with ethylacetate, stirred for 15 min and filtered. The remaining solid was dried under vacuum.

Yield: 0.77 g (78 %)

^{1}H-NMR (DMSO-d_6): 12.35 (s, 1H), 8.18-8.3 (m, 2H), 7.9-8.15 (m,4H), 7.79 (d, J=8Hz, 2H), 7.61 (d, J=8Hz, 2H), 7.05 (d, J=8Hz, 2H), 4.42-4.65 (m, 2H), 4.1 (q,

J=7Hz, 2H), 3.24-3.6 (m, 4H), 2.88-3.05 (m,1H), 2.02-2.35 (m, 2H), 1.38 (t, J=7Hz, 3H)

The compound of Example 16 was separated into its pure enantiomers by HPLC on a Kromasil 100-5-CHI-DMB (5µm) column using a mixture consisting of n-heptane : (0.2% acetic acid/TBME) : dichloromethane (40 : 54 : 6 by volume) as eluant.

Example 17

(+)-4-(4'-Ethoxy-biphenyl-4-yl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-4-oxo-butyric acid

First eluting enantiomer A
Yield: 168 mg (24 %)
$[\alpha]_D^{20}$(c= 0.66g/100ml, THF) = +17.05°

Example 18

(-)-4-(4'-Ethoxy-biphenyl-4-yl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-4-oxo-butyric acid

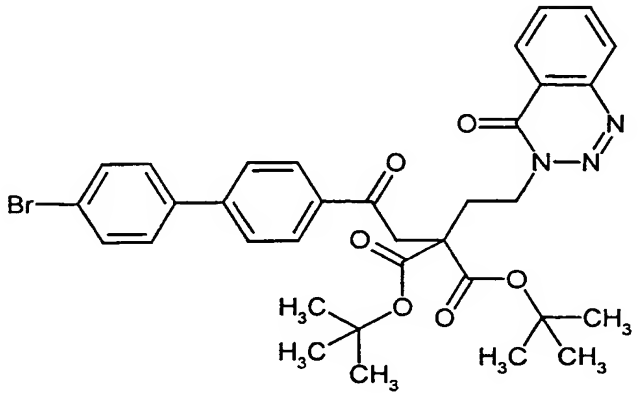
Second eluting enantiomer B:
Yield: 153 mg (22%)
$[\alpha]_D^{20}$(c= 0.58g/100ml, THF) = -18.78°

Example 19

4-(4'-Bromo-biphenyl-4-yl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-4-oxo-butyric acid

Intermediate 19A

tert.-Butyl-4-(4'-bromo-biphenyl-4-yl)-4-oxo-2-(tert.-butyloxycarbonyl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-butanoate



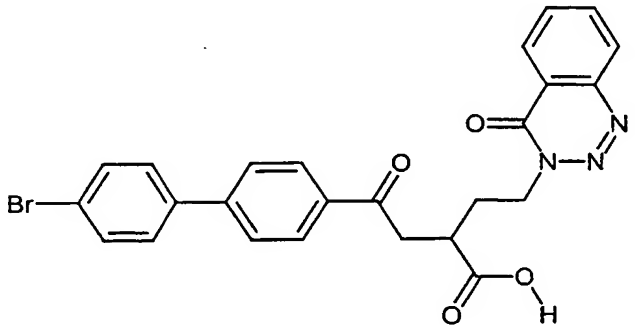
A solution of Intermediate 13C (1.27 g, 3.25 mmol) in 15 mL DMF was added dropwise to a suspension of NaH (160 mg, 4.06 mmol) in 5 mL DMF and stirred for 30 min at rt. 2-Bromo-4'-(4'-bromo-phenyl) acetophenone (1.15 g, 3.25 mmol) in 15 ml DMF was added slowly and the resulting mixture was stirred for 2.5 h at rt. The reaction was quenched with saturated NH_4Cl solution, extracted twice with diethyl ether, washed with saturated $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated. The crude product was purified using flash chromatography (Hexane / Ethyl acetate : 9/1-> 3/1).

Yield: 1.27 g (58 %)

^{1}H-NMR (DMSO-d_6): 7.88-8.25 (m, 6H), 7.81 (d, J=8Hz, 2H), 7.7 (s, 4H), 4.39-4.51 (m, 2H), 3,75 (s, 2H), 2.45-2.58 (m, 2H), 1.4 (s, 18H)

Example 19

(rac)-4-(4'-Bromo-biphenyl-4-yl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-4-oxo-butyric acid



1.24 g (1.87 mmol) of Intermediate 19A was added in one portion to a cooled (0°C) 1:1 mixture of CH_2Cl_2 and trifluoroacetic acid (20 mL). The reaction mixture was stirred for 2 h at rt, evaporated and dried under vacuum. The residue was disolved in 20 mL dioxane and heated for 5 h under reflux. The rection mixture was evaporated, the residue triturated with ethylacetate, stirred for 15 min and filtered. The remaining solid was dried under vacuum.

Yield: 0.77 g (78 %)

^{1}H-NMR (DMSO-d_6): 12.4 (s, 1H), 7.9-8.3 (m, 6H), 8579 (d, J=8Hz, 2H), 7.72 (s, 4H), 3.28-3.62 (m, 4H), 2.88-3.05 (m,1H), 2.02-2.35 (m, 2H)

700 mg of the compound of Example 19 were separated into the pure enantiomers by HPLC on a Kromasil 100-5-CHI-DMB (5µm) column using a mixture consisting of n-heptane : (0.2% acetic acid/TBME) : dichloromethane (40 : 54 : 6 by volume) as eluant.

Example 20

(+)-4-(4'-Bromo-biphenyl-4-yl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-4-oxo-butyric acid

First eluting enantiomer A:

Yield: 265mg (38%)

$[\alpha]_D^{20}$(c=0.61g/100ml, THF) = +14.34°

Example 21

(-)-4-(4'-Bromo-biphenyl-4-yl)-2-[2-(4-oxo-4H-benzo[d][1,2,3]triazin-3-yl)-ethyl]-4-oxo-butyric acid

Slower eluting enantiomer B:

Yield: 219mg (31%)

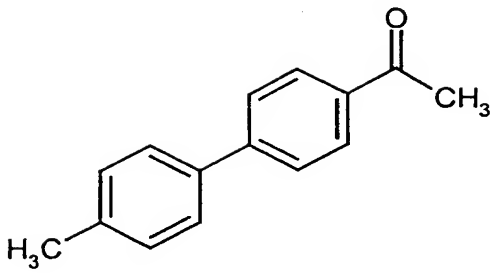
$[\alpha]_D^{20}$(c=0.62 g/100ml, THF) = -14.32°

Example 22

(rac)-4-(4'-methyl[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}butanoic acid

Intermediate 22A

1-(4'-methyl[1,1'-biphenyl]-4-yl)-1-ethanone



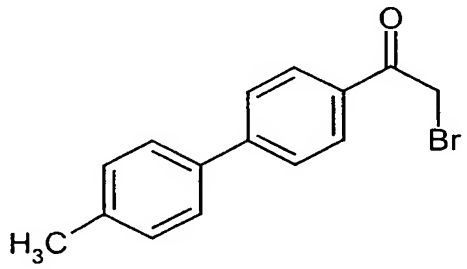
20 g (119 mmol) 4-methylbiphenyl and 19.81 g (149 mmol) aluminiumchloride are dissolved in 120 ml dichloromethane. At a temperature below 20°C 9.38 g (119 mmol) acetylchloride is added dropwise and the reactionmixture is stirred at roomtemperature for 2hrs. The solution is added to a mixture of 200 ml ice water and 55 ml conc. HCl, the organic phase is separated, dried and concentrated. The residue was purified by chromatography (cyclohexane/ethylacetate=10:1).

Yield: 11.5g (46%).

200 MHz ^{1}H-NMR ($CDCl_3$): 2.41, s, 3H; 2.62, s, 3H; 7.29, d, 2H; 7.53, d, 2H; 7.68, d, 2H; 8.02, d, 2H.

Intermediate 22B

2-bromo-1-(4'-methyl[1,1'-biphenyl]-4-yl)-1-ethanone



4.76 g (22.6 mmol) of Intermediate 22A are dissolved in a mixture of 50 ml ether and 50 ml methanol with gentle heating. The solution is treated with 3.06 g (29.4 mmol)

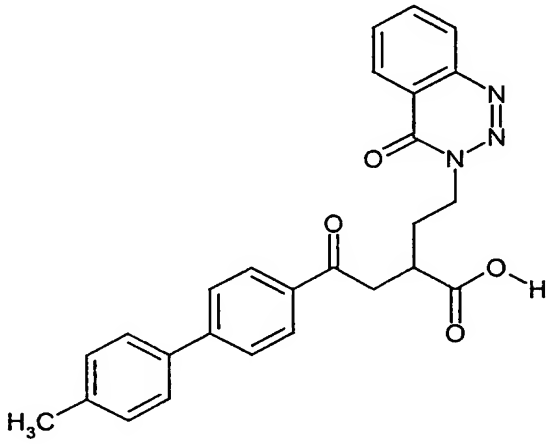
boronicacidtrimethylester at roomtemperature. 4.16 g (26 mmol) bromine are added dropwise at 0°C. The reaction mixture is stirred until disappearance of the yellow colour. The solvents are removed in vacuo and the product obtained by triturating with ether.

Yield: 4.69g (72%)

200 MHz ^{1}H-NMR ($CDCl_3$): 2.41, s, 3H; 4.47, s, 2H; 7.29, d, 2H; 7.53, d, 2H; 7.70, d, 2H; 8.06, d, 2H.

Example 21

(rac)-4-(4'-methyl[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}butanoic acid



To a suspension of 0.257 g (6.42 mmol) sodiumhydride (60% in mineral oil) in 20 ml DMF under argon is added dropwise a solution of 2.0g (5.14 mmol) of Intermediate 13C in 20 ml DMF. After stirring for 30 minutes at room temperature 1.49 g (5.14 mmol) Intermediate 21B in 20 ml DMF are added dropwise. The reaction mixture is stirred at room temperature for 2.5 hrs and poured onto 150 ml saturated NH_4Cl-solution. The aqueous phase is extracted twice with ethylacetate, the combined organic phases are dried and solvents removed in vacuo. The residue is

purified by chromatography (cyclohexan/ethylacetate:10:1), to give 1.87 g (61%) di(tert-butyl)2-[2-(4'-methyl[1,1'-biphenyl]-4-yl)-2-oxoethyl]-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}malonate.

0.46 g (0.77 mmol) di(tert-butyl)2-[2-(4'-methyl[1,1'-biphenyl]-4-yl)-2-oxoethyl]-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}malonate is dissolved in a mixture of 4ml dichloromethane and 4ml trifluoroaceticacid and the resulting solution is stirred for 2 hrs at room temperature. 10ml toluene are added and the solvents are removed in vacuo. The residue is taken up in 8ml 1,4-dioxane and the resulting solution refluxed for 6 hrs and stirred at room temperature for 12hrs. The solvent is removed in vacuo and the product obtained by trituration with ethylacetate.

yield: 0.233g (65%)

200 MHz ^{1}H-NMR ($CDCl_3$+DMSO): 2.22, m, 1H; 2.40, s, 3H; 2.70-3.02, m, 2H; 3.29, m, 1H; 3.54, m, 1H; 4.63, t, 2H; 7.28, d, 2H; 7.55, d, 2H; 7.65, d, 2H; 7.78-8.04, m, 4H; 8.16, d, 1H; 8.33, d, 1H.

The compound of Example 22 was separated into its pure enantiomers by HPLC on a Kromasil 100-5-CHI-DMB (5µm) column using a mixture consisting of i-heptane : dichloromethane : acetic acid (480 : 40 : 1 by volume) as eluant.

Example 23

(+)-4-(4'-methyl[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}butanoic acid

First eluting enantiomer A:

$[\alpha]_D^{20}$(c=0.88 g/100ml, THF) = +15.2°

Example 24

(-)-4-(4'-methyl[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}butanoic acid

Second eluting enantiomer B

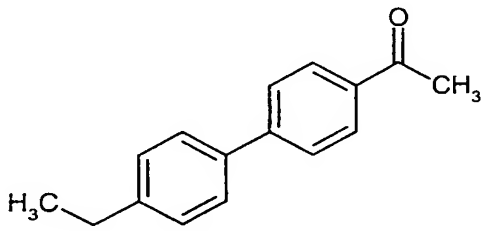
$[\alpha]_D^{20}$(c=0.93 g/100ml, THF) = -13.2°

Example 25

(rac)-4-(4'-ethyl[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}butanoic acid

Intermediate 25A

1-(4'-ethyl[1,1'-biphenyl]-4-yl)-1-ethanone



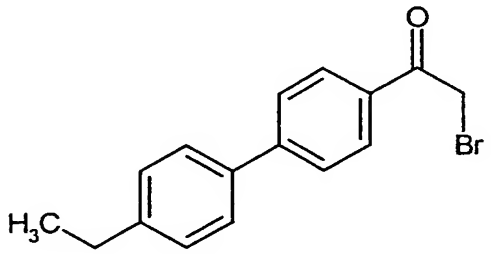
10 g (55 mmol) 4-ethylbiphenyl and 9.15 g (169 mmol) aluminiumtrichloride are dissolved in 60 ml nitrobenzene. At a temperature below 20°C 4.31 g (55 mmol) acetylchloride is added dropwise and the reaction mixture is stirred at room temperature for 2 hrs. The solution is added to a mixture of 80 ml ice water and 14 ml conc. HCl, the organic phase is separated, dried and concentrated. The product is obtained by trituration with petrolether.

Yield: 8.8g (71%).

200 MHz ^{1}H-NMR ($CDCl_3$): 1.29, t, 3H; 2.52, s, 3H; 2.72, quart., 2H; 7.30, d, 2H; 7.58, d, 2H; 7.69, d, 2H; 8.02, d, 2H.

Intermediate 25B

2-bromo-1-(4'-ethyl[1,1'-biphenyl]-4-yl)-1-ethanone



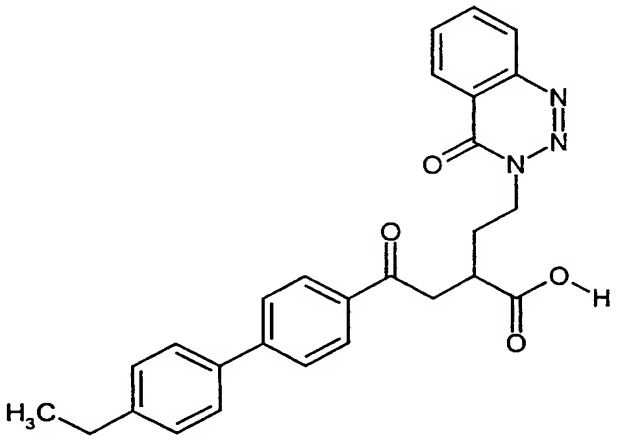
3.45 g (15.4 mmol) of Intermediate 25A are dissolved in a mixture of 70 ml methanol and 40 ml ethanol with gentle heating. The solution is treated with 2.08 g (20mmol) boronicacidtrimethylester at roomtemperature. 2.7 g (20 mmol) bromine are added dropwise at 0°C. The reaction mixture is stirred until disappearance of the yellow colour. The solvents are removed in vacuo and the product obtained by triturating with ether.

Yield: 3.74g (76%)

200 MHz ^{1}H-NMR ($CDCl_3$): 1.29, t, 3H; 2.72, quar., 2H; 4.47, s, 2H; 7.32, d, 2H; 7.57, d, 2H; 7.70, d, 2H; 8.04, d, 2H.

Example 25

(rac)-4-(4'-ethyl[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}butanoic acid



To a suspension of 0.330 g (8.25mmol) sodiumhydride (60% in mineral oil) in 20 ml DMF under argon is added dropwise a solution of 2.57g (6.6 mmol) of Intermediate 13C in 20 ml DMF. After stirring for 30 minutes at room temperature 2.0 g (6.60 mmol) of Intermediate 25B in 20 ml DMF are added dropwise. The reaction mixture is stirred at room temperature for 2.5 hrs and poured onto 150 ml saturated NH_4Cl-solution. The aqueous phase is extracted twice with ethylacetate, the combined organic phases are dried and solvents removed in vacuo. The residue is purified by chromatography (cyclohexan/ethylacetate:10:1), to give 3.44 g (85%) di(tert-butyl)2-[2-(4'-ethyl[1,1'-biphenyl]-4-yl)-2-oxoethyl]-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}malonate.

3.44 g (5.62 mmol) di(tert-butyl)2-[2-(4'-ethyl[1,1'-biphenyl]-4-yl)-2-oxoethyl]-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}malonate is dissolved in a mixture of 30 ml dichloromethane and 30 ml trifluoroaceticacid and the resulting solution is stirred for 2 hrs at room temperature. 10 ml toluene are added and the solvents are removed in vacuo. The residue is taken up in 60 ml 1,4-dioxane and the resulting solution re-

fluxed for 6 hrs and stirred at room temperature for 12hrs. The solvent is removed in vacuo and the product obtained by trituration with ethylacetate.

yield: 1.77g (67%)

200 MHz ^{1}H-NMR (DMSO-d_6): 1.22, t, 3H; 2.02-2.33, m, 2H; 2.67, quar., 2H; 2.97, m, 1H; 3.26-3.62, m, 2H; 4.53, t, 2H; 7.35, d, 2H; 7.69, d, 2H; 7.82, d, 2H; 7.89-8.13, m, 4H; 8.23, t, 2H; 12.37, s, 1H.

The compound of Example 25 was separated into its pure enantiomers by HPLC in analogy to Example 21.

Example 26

(+)-4-(4'-ethyl[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]ethyl}butanoic acid

First eluting enantiomer A:

$[\alpha]_D^{20}$(c=0.91 g/100ml, THF) = +14.4°

Example 27

(-)-4-(4'-ethyl[1,1'-biphenyl]-4-yl)-4-oxo-2-{2-[4-oxo-1,2,3-benzotriazin-3(4H)-yl]-ethyl}butanoic acid

Second eluting enantiomer B:

$[\alpha]_D^{20}$(c=0.91 g/100ml, THF) = -16.2°